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All correspondence concerning manuscripts, editorial matters and subscription should be addressed to:

Acta Dermato-Venereologica
S:t Johannesgatan 22, SE-753 12 Uppsala, Sweden

Editorial Manager, Mrs Agneta Andersson
Please send an E-mail

Editorial Assistant: Ms Anna-Maria Andersson
Please send an E-mail

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Advances in dermatology and venereology

BLISTERING SKIN DISORDERS

Theme Editor:

Kaisa Tasanen

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REVIEW ARTICLE

Skin Fragility: Perspectives on Evidence-based Therapies

Leena BRUCKNER-TUDERMAN
Department of Dermatology, Medical Faculty and Medical Center – University of Freiburg, Freiburg, Germany

The term skin fragility disorders describes a group of conditions in which the structural integrity of the skin is compromised and its resistance to external shear forces diminished. Skin fragility can have different causes, ranging from genetic variations to inflammatory or physical phenomena. The genetic skin fragility disorders, collectively called epidermolysis bullosa, serve as a paradigm for the study of causes and mechanisms of skin fragility. Recent biomedical research has revealed substantial genetic heterogeneity of the epidermolysis bullosa group, delivered ample new knowledge on its pathophysiology, and facilitated the design of evidence-based therapeutic strategies. The therapy development process extends from *in vitro* testing to preclinical validation in animal models, and clinical trials. This article reviews different approaches to curative and symptom-relief therapies, and appraises their status and perspectives for clinical implementation.

Key words: skin blistering; genodermatosis; molecular therapy; symptom-relief.

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Corr: Leena Bruckner-Tuderman, Department of Dermatology, Medical Faculty and Medical Center – University of Freiburg, Hauptstrasse 7, DE-79104 Freiburg, Germany. E-mail: bruckner-tuderman@uniklinik-freiburg.de

SIGNIFICANCE

The term skin fragility describes skin that blisters and breaks easily upon mild friction or trauma. Skin fragility can have many causes, ranging from genetic variants to a compromised immune system, infections or adverse drug reactions. Studies of genetic skin fragility disorders, such as epidermolysis bullosa, have provided better understanding of their causes and mechanisms. At least 20 genes may be involved in epidermolysis bullosa, and secondary phenomena, such as inflammation or fibrosis, can worsen the disease. No cure is yet available, but international research is developing novel approaches to cure the disease and alleviate its symptoms. This article reviews these new developments and appraises their clinical implementation.

The term skin fragility refers to pathologically altered skin that blisters and breaks easily upon mild friction, pressure or trauma. The breakage can occur in different skin layers, within the epidermis, along the dermal–epidermal junction, or in the upper dermis. The factors that can cause skin fragility and blistering range from genetic variations to (auto)immune, inflammatory, physical, mechanical, infectious, or drug-induced processes. Correspondingly, many classes of disorders can be described using this term, and the differential diagnosis is broad (1) (**Table I**). As a genetic skin fragility disorder, epidermolysis bullosa (EB) serves as a useful paradigm for these disorders, and research into EB has delivered new information about the pathophysiology of skin fragility that is clinically relevant (2, 3). For example, molecular characterization of autoantigens in acquired blistering diseases has led to the development of molecular diagnostic tests that are in standard use in diagnostics, management and monitoring of autoimmune bullous disorders (4, 5).

Table I. Differential diagnosis of skin fragility

- Genetic skin fragility disorders
- Autoimmune blistering disorders
- Skin fragility induced by infections
- Skin fragility induced by acute inflammation
- Metabolic conditions with blisters
- Bullous drug reactions
- Mechanically induced skin blisters
- Physically induced skin fragility

EPIDERMOLYSIS BULLOSA AS A PARADIGMATIC SKIN FRAGILITY DISORDER

EB has been studied intensively, and the genetic causes and disease mechanisms of the different EB types are rather well understood (1–3). The initial simple assumption that a single pathogenic gene variant/mutation explains all symptoms still holds true in principle. However, the complexity of cellular and molecular processes unleashed by mechanical stress on EB skin is far greater than anticipated; a fact that has major consequences for the design and development of therapies.

As background for the discussion and appraisal of therapy developments, a short introduction to EB, its current diagnostics and management follows.

Epidermolysis bullosa classification

The EB group encompasses 4 main types: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler syndrome (6) (**Table II**). The division into types is based on the morphological level of separation

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Table II. Major types of epidermolysis bullosa (EB)

EB types (abbreviation)	Level of blistering
Epidermolysis bullosa simplex (EBS)	Intra-epidermal
Junctional epidermolysis bullosa (JEB)	Along the basement membrane
Dystrophic epidermolysis bullosa (DEB)	In the upper dermis
Kindler syndrome (KS)	Mixed*

*The blistering can occur at any or all of the above levels.

within the dermal–epidermal junction zone. In EBS, the blisters form within the epidermis, in JEB within the basement membrane, and in DEB just below the basement membrane. In Kindler syndrome, blisters can form at all levels. A common hallmark for all EB types is trauma-induced skin blistering and fragility, but each of them contains a number of subtypes, in which the extent of skin lesions and the associated organ manifestations can vary to a great extent (**Fig. 1**). In April 2019, an international EB consensus classification meeting took place in London. Experts from all over the world updated and revised the consensus classification; the new classification paper is in preparation (6). The main changes are related to the EBS group that has expanded significantly in the past 5 years. Some of the very severe forms in this group, but also mild disorders with minimal skin fragility, were clearly regarded as skin fragility disorders, but not as EB. The new classification includes for the first time syndromal EB subtypes with multi-organ involvement.

Modern diagnostics of epidermolysis bullosa

A well-defined diagnosis, with as much molecular precision as possible, is recommended for all patients with EB. A clear diagnosis facilitates disease management, including prognostication and genetic counselling (7, 8). Furthermore, as novel therapies emerge, molecular diagnosis is often a prerequisite for inclusion in clinical trials; it will also be needed for application of future personalized therapies (8). The recommended diagnostic procedure involves immunofluorescence mapping of a skin biopsy as a first step; this enables identification of the blistering level and definition of candidate gene(s) for subsequent genetic analysis. In cases with inconclusive clinical presentation, genetic diagnostics using next generation sequencing (NGS) technologies, such as EB gene panel-based diagnostics or clinical exome analysis, are recommended (7).

Current disease management

Since there is currently no cure for EB, a combination of symptomatic treatment modalities is used, depending on needs. Protection from trauma, cleaning, disinfecting, and moisturizing the skin belong to daily basic measures. Different wound management modalities are defined in guidelines (http://www.debra-international.org/clinical-guidelines). Furthermore, since involvement of other organs is common in more severe EB, and since chronic skin fragility and painful wounds diminish the quality of life of the affected individuals and their families, interdisciplinary and multi-professional management, including psychosocial care, are highly recommended (www.debra-international.org/clinical-guidelines/complete-eb-guidelines.html).

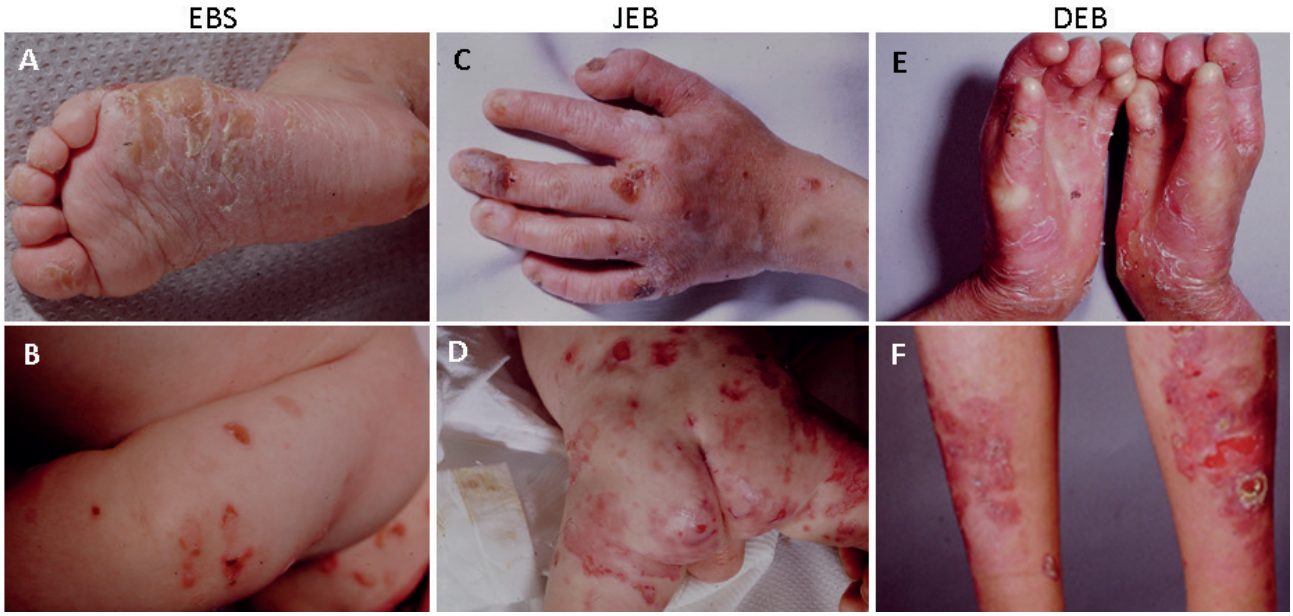

Fig. 1. Typical clinical presentations in different types of epidermolysis bullosa (EB). (A, B) EB simplex (EBS). (A) Blisters, erosions and scaling in the foot of a 2-year-old child. (B) Disseminated blisters on the trunk and extremities of a newborn. (C, D) Junctional EB (JEB). (C) Blisters, erosions and loss of nails in the hand of a 7-year-old girl with moderate JEB. (D) Typical extensive skin fragility in the buttocks area and back of a newborn with severe generalized JEB. (E, F) Dystrophic EB (DEB). (E) Strong scarring and fusion of digits in the hand of an 8-year-old girl with severe generalized DEB. (F) Trauma-induced blistering, inflammation and scarring on the shins of a 12-year-old girl with moderate DEB.

Expert centres and European Reference Networks

Numerous expert centres for EB exist worldwide. Most of these are members of the EB-Clinical Network "EB-Clinet" (www.EB-Clinet.org), which works together with the patient groups (www.debra-international.org). The centres provide information and advice to patients and caregivers, as well as services ranging from diagnostics to genetic counselling and interdisciplinary management plans. In 2017, the European Commission launched European Reference Networks (ERNs) for rare diseases for high-quality diagnostics, management, and research. The goal is to tackle complex or rare diseases with a concentration of knowledge and resources (https://ec.europa.eu/health/ern). The ERNs provide a dedicated IT platform, telemedicine tools and a virtual advisory board of specialists from different disciplines to evaluate the diagnosis of a patient and plan the treatment. An important principle is that the medical knowledge and expertise "travel", and not the patients, who should have the comfort of staying at home in their supportive environment. ERN-Skin encompasses 56 healthcare providers from 18 countries who are endorsed by their national authorities and committed to pool their knowledge and expertise within the framework of the ERN-Skin (https://ern-skin.eu/). Two approaches are taken: (*i*) a disease approach with 8 sub-thematic groups on high-level patient management and research; (*ii*) a transversal approach focusing on teaching and training, E-health, registries and research, deep phenotyping and clinical outcomes. One of the 8 sub-thematic groups deals with EB.

EMERGING NOVEL THERAPY APPROACHES

Despite all the structural developments in the field of rare skin diseases, the unmet medical need remains high, and novel evidence-based therapies are urgently needed. Development of new treatments is strongly promoted by patient advocacy groups, which are very active in setting priorities and funding patient-oriented research (www.debra-international.org; www.ebresearch.org/, www.cure-EB.org).

As the therapeutic era for skin fragility disorders progresses it becomes clear that therapy strategies with "intention to cure" are far more complex and difficult than expected. Gene therapy development faces technological challenges with vectors, targeting skin stem cells, achieving long-term therapeutic effects, etc. Therefore, a variety of methodologies relating to gene replacement, gene editing, and modifying transcription and translation are being tested. Because patients demand more rapid development of treatments that bring relief, the focus has turned to so-called symptom-relief and regenerative therapies that, although they do not bring cure, will alleviate symptoms, offer relief and improve quality of life. The therapies that have reached a clinical

Table III. Currently recruiting clinical therapy trials for epidermolysis bullosa (EB) (as of June 2019)

Therapy type	Investigational drug	EB type	Trial identification number
Therapies with curative aim			
Gene therapy	Transplantation surgery of genetically corrected cultured epidermal autograft	JEB with COL17A1 mutations	ClinicalTrials.gov ID: NCT03490331
	Genetically corrected cultured epidermal autograft	RDEB*	ClinicalTrials.gov ID: NCT02984085
	FCX-007, Genetically modified autologous human dermal fibroblasts	RDEB*	ClinicalTrials.gov ID: NCT02810951
	KB103, topically applied non-integrating, replication-incompetent herpes simplex virus vector expressing human collagen VII protein.	DEB	ClinicalTrials.gov ID: NCT03536143
Antisense oligonucleotides	QR-313, topically applied antisense oligonucleotide	DEB with mutations in exon 73 of COL7A1	ClinicalTrials.gov ID: NCT03605069
PTC read-through	Gentamicin, intravenous	RDEB*	ClinicalTrials.gov ID: NCT03392909
	Gentamicin, topical	JEB	ClinicalTrials.gov ID: NCT03526159
Protein therapy	PTR-01, recombinant human collagen VII	RDEB*	ClinicalTrials.gov ID: NCT03752905
Regenerative cell-based therapies			
Cell therapy	Serial mesenchymal stem cell (MSC) infusions from a related donor	All EB types	ClinicalTrials.gov ID: NCT02582775
	Allogeneic stem cell transplantation and "off-the-shelf" mesenchymal stem cells	All EB types	ClinicalTrials.gov ID: NCT01033552
	Allogeneic ABCB5-positive stem cells	RDEB*	ClinicalTrials.gov ID: NCT03529877
	Epidermal grafts generated using the Cellutome System	EB after hematopoietic cell transplantation	ClinicalTrials.gov ID: NCT02670837
Symptom-relief therapies			
Anti-fibrotic	Losartan, systemic	RDEB	EudraCT No.: 2015-003670-32
Anti-inflammatory	Pharmacokinetics, safety of diacerein after maximum use	EBS	ClinicalTrials.gov ID: NCT03472287
	Oleogel-S-10, topical	All EB types	ClinicalTrials.gov ID: NCT03068780
	BPM31510 3.0% cream, topical	All EB types	ClinicalTrials.gov ID: NCT02793960
	Sirolimus, topical	EBS	ClinicalTrials.gov ID: NCT03016715
Accelerator of wound healing	RGN-137, a thymosin beta-4 gel, topical	JEB, DEB	ClinicalTrials.gov ID: NCT03578029
	Amniotic membrane	RDEB	ClinicalTrials.gov ID: NCT02286427
Analgesic	Ropivacaine, topical	All EB types	ClinicalTrials.gov ID: NCT03730584
Anti-pruritic	Neurokinin-1 receptor Antagonist, oral	All EB types	ClinicalTrials.gov ID: NCT03836001
Anti-hidrotic	Botulinum toxin	EBS	ClinicalTrials.gov ID: NCT03453632

EBS: EB simplex; JEB: junctional EB; DEB: dystrophic EB; RDEB; recessive DEB.

trial stage and are recruiting trial participants are summarized in **Table III**.

Gene therapies

Retrovirus-mediated gene correction in keratinocytes and subsequent grafting of gene-corrected epidermal sheets was developed many years ago as a principally valid method to treat JEB or DEB skin (9, 10 and references therein). Recently, this method was used to replace approximately 80% of the skin surface in a very severely ill child with JEB (9, 10). A similar approach is being tested in DEB for maintenance of wound healing (11). So far, 7 patients with RDEB have been treated with *COL7A1*-gene corrected keratinocyte grafts, many of them have durable wound-healing (www.abeonatherapeutics. com). However, the classical gene therapy approaches still deal with technological issues relating to vector safety and to optimal transfection/transduction efficiency of stem cells. Gene editing using the CrispR/Cas technology has shown promise in correcting *COL7A1* mutations in RDEB keratinocytes (12) and RDEB fibroblasts (13) *in vitro* and at a preclinical level. Further research strategies encompass approaches with gene-corrected iPS cells (14–17). A newly introduced technology employs a non-integrating, replication-incompetent herpes simplex virus 1 (HSV-1) vector expressing human collagen VII (www.krystalbio.com). The vector preferably targets keratinocytes/epidermis, and a pilot trial using topical treatment of DEB addresses wound-healing as a primary outcome marker (Table III).

Natural gene therapy

The term "natural gene therapy" describes revertant mosaicism, i.e. the spontaneous conversion of a somatic cell with a mutation and pathological phenotype into a cell that has acquired a second, compensating mutation and gained a normal phenotype (18). Revertant mosaicism is relatively common in genetic disorders, and in most classic EB types revertant mosaic skin patches can be found by a well-trained expert. Approximately 5 years ago, the first "natural gene therapy"-based treatment of EB was reported, JEB skin was transplanted with small split-thickness revertant grafts (19). More recently, cultured epidermal autografts generated from clinically revertant skin were applied to treat DEB wounds in 3 patients. The take was 55–87%, and the clinical effects remained for at least 76 weeks of follow-up (20).

RNA-based therapies

Different approaches can be used to skip or replace exons at the RNA level. In an ex vivo RNA trans-splicing-based approach 7 exons were replaced, including the one with a *KRT14* mutation, to correct the cellular phenotype in EBS keratinocytes. The corrected keratinocytes formed a stable epidermis in a xenograft model, indicating that trans-splicing-mediated RNA therapy could have potential for clinical implementation (21). Another option is to employ antisense oligonucleotides to skip the mutated exon in the transcription process. Subsequently, a polypeptide that lacks the amino acid sequence encoded by the skipped exon is synthesized; this is usually at least partly functional. Collagenopathies are particularly suitable for this approach, since exons of collagen genes are typically in-frame and small. Their deletion is not likely to cause major structural changes in the affected protein. Of the EB genes, the collagen VII gene is interesting, since exon 73 harbours a high number of mutations. *In vitro* experiments showed that antisense oligonucleotide-induced skipping of exon 73 leads to a partially functional collagen VII that could potentially improve DEB skin functions (22, 23). A phase 1/2 multicentre clinical trial plans to test this approach in DEB patients carrying specific mutations (www.wings-tx.com).

Premature termination codons read-through

The idea of read-through of premature termination codons (PTC) arose from the knowledge that nonsense-mediated mRNA decay is often caused by PTC (24). Overriding the mutation during transcription would presumably generate a full-length translation product, i.e. a polypeptide with a minor modification that is likely to be adequately functional. Aminoglycoside antibiotics induce PTC read-through. However, the neighbouring nucleotides of the mutations influence the efficiency of the read-through and, therefore, not all PTC are suitable for aminoglycoside treatment. Gentamicins suppressed *COL7A1* and *LAMB3* mutations with some efficacy in vitro and *in vivo* (25, 26). Human therapy trials assess the suitability and tolerability of intravenous gentamicin in RDEB and topical gentamicin in JEB (Table III). A challenge with this category of drugs is the spectrum of adverse effects, such as renal and ototoxicity, or potency to induce contact sensitization. Gentamicin B1, a minor gentamicin constituent, has been suggested to be superior in this context due to its high potency to suppress PTC and its low toxicity (27). Amlexanox, an anti-inflammatory drug, can also induce PTC read-through. *In vitro*, in collagen VII-negative DEB cells with PTC mutations, it induced collagen VII protein production (28).

Protein therapy

Protein therapies, in particular enzyme replacements, have been designed and tested for several inborn errors of metabolism (29). In case of EB, the challenge is that many of the proteins that are mutated and/or missing (collagens, laminins, keratins) are large and, by the nature of their physiological functions, have a tendency to

form aggregates. These characteristics do not facilitate intravenous administration and homing of the protein to the required site of action. With this background it seems surprising that intravenous and intradermal injections of recombinant collagen VII in DEB model mice resulted in homing of some collagen into the skin and the dermal–epidermal junction, without major adverse effects (30). A clinical trial is currently testing the safety of recombinant collagen VII in RDEB (Table III; http://phoenixtissuerepair.com).

DISEASE-MODIFYING APPROACHES

With increasing experience in preclinical and clinical development of therapies for EB, the complexity of treatment-related issues has surprised most scientists (8, 31). We realize that curative therapies will need many years to enter the clinics and, at the same time, the pressure from patients for treatments increases. The scientific community has reacted by searching for possibilities to modify disease activity and to alleviate symptoms. The rationale for such symptom-relief approaches comes from basic research on disease mechanisms in EB. Many *in vitro* and preclinical studies have laid the foundation for using cells or targeting, for example, cytokines or growth factors that drive EB phenotypes (8). The goal of these treatments is to improve functions of the skin and make the patients feel better. Three groups of symptom relief therapies are delineated below: (*i*) regenerative cell-based therapies; (*ii*) topical pharmacological therapies; and (*iii*) systemic therapies with biomolecules and repurposed drugs.

Regenerative cell-based therapies

From many different angles, cell therapies for EB have turned out to be more challenging than initially expected. They are very unlikely to bring cure, and have recently been re-grouped into the category of disease-modifying treatments. Currently, both local and systemic applications are being tested for disease-modifying capacity.

Intradermal cell injections

Early investigations with intradermal injections of fibroblasts or human bone marrow-derived mesenchymal stem cells into RDEB mice demonstrated that the cells produced collagen VII that homed into the dermal–epidermal junction and ameliorated its stability (32–34). However, in humans the tolerability and efficacy of this therapeutic approach were poorer than expected. The injections were very painful and improvement of the skin very limited (35). One study observed a comparable improvement of wound healing in DEB, regardless of whether fibroblasts or vehicle was injected (36). Recently, the approach has been modified with the use of gene-corrected fibroblasts that produce large amounts of collagen VII. Preliminary information indicates that the injections bring some *de novo* collagen VII into the treated areas, but the full potential of this approach remains to be seen (37; www.fibrocell.com).

Systemic stem cell therapies

Bone marrow transplantation has been tested as treatment for different genetic diseases, including severe DEB (38). Disappointingly, the therapeutic effect and duration were not as positive as hoped for and, as is well known, the complications of bone marrow transplantation can be life-threatening (39). Subsequently, different conditioning regimens have been tested, most recently a regimen that combines reduced-intensity conditioning, post-transplant cyclophosphamide and infusions of immunomodulatory allogeneic mesenchymal stromal cells (40). Treatment of children with RDEB with intravenously administered human allogeneic mesenchymal stem cells made them feel better, but brought no collagen VII into the skin (41). The efficacy of an ABCB5-positive subpopulation of mesenchymal stem cells for symptom-relief in adults with RDEB is assessed in a current trial (*www.rheacell.com*). In addition, cord-blood derived stem cells have shown some potential as systemic anti-fibrotic treatment in a preclinical setting (42).

Topical pharmacological therapies

Diacerein from rhubarb root extracts has been implicated as possible treatment for EBS skin (43, 44). The rationale involves the capacity of diacerein to dampen the inflammatory response caused by epidermal cell rupture in EBS (43). The cell disruption is a consequence of keratin 5 and 14 mutations that cause intermediate filament aggregation and loss of stabilization by the cytoskeleton. *In vitro* data demonstrated both anti-inflammatory properties of diacerein and its potential for stabilizing EBS cells, then a pilot clinical trial demonstrated fewer blisters in diacerein cream-treated skin in part of the study population (44).

Wound-healing in EB can be supported by another plant-derived compound with anti-inflammatory properties, namely betulin-based oleogel isolated from birch bark. Betulin was shown to support keratinocyte differentiation (45), enhance re-epithelialization and facilitate wound healing *in vitro* and *in vivo* (46, 47). An ongoing placebo-controlled phase 3 study assesses the efficacy of oleogel in patients with EB, regardless of subtype (48).

Systemic disease modifying therapies

Anti-inflammatory approaches. Recent basic research, followed by preclinical and clinical validation, has revealed an unanticipated role for inflammatory cascades in EB. In EBS, keratin mutations and keratinocyte fragility induce expression of specific cytokines and T-cell-

mediated inflammatory responses, which manifest with itch as a bothersome symptom (49, 50). A vicious circle is generated by itch, scratching and subsequent skin blistering, which leads to a stronger inflammatory response. Although non-specific anti-inflammatory therapies with NSAIDs are not beneficial, first pilot studies with specific systemic treatments show promise. For example, anti-IL17 interval therapy with apremilast worked well in 3 individuals with of EBS (50).

Antifibrotic therapy approaches. Based on an ample body of scientific literature, severe DEB can be regarded as a systemic disease, since systemic inflammation is prominent and the secondary progressive fibrosis affects many organs (51). Therefore, drugs that inhibit inflammation and fibrosis could potentially relieve symptoms in DEB, such as inflammation-caused itch or formation of strictures and contractures, including fusion of digits.

A repurposed drug, losartan, has shown such benefits in DEB on the preclinical level (52). This drug for treatment of high blood pressure also has anti-fibrotic potential in some disease constellations. The mechanism is based on its ability to inhibit TGFβ signalling via AT-1 receptor antagonism (52). Since inflammation and hyper-active TGFβ signalling contribute to DEB-associated fibrosis in a major manner (8, 53, 54), losartan appeared suitable as treatment. The expectations were met in losartan-treated RDEB model mice, inflammation and TGFβ activity were reduced, progression of fibrosis inhibited and fusion of digits delayed (53). As a logical next step, a clinical trial currently assesses safety and tolerability of losartan in children with moderate-to-severe DEB. The study is also likely to generate preliminary information on the ability of losartan to alleviate symptoms in human DEB (Table III).

Another modulator of TGFβ signalling is the small leucine-rich proteoglycan decorin. Endogenous decorin levels are known to correlate with clinical severity in RDEB (55). In a preclinical study, systemic administration of lentivirally overexpressed human decorin reduced TGFβ levels and fibrotic traits, and enhanced survival of the RDEB mice (56). These observations indicate that extracellular matrix biomolecules modulating TGFβ signalling may have potential for systemic anti-fibrotic therapy for DEB.

In addition to the above small (bio)molecules, a high mobility group box 1 (HMGB1)-derived peptide may improve systemic fibrosis in DEB. HMGB1 has variable functions and has been implicated in both physiological and pathological processes (57). In the context of EB, its relevance lies in its ability to release a specific anti-inflammatory population of mesenchymal stem cell from the bone marrow into the circulation and from there into damaged skin (58). First treatments of RDEB mice with a HMGB1-derived peptide resulted in improvement of skin fibrosis and gastrointestinal strictures (K. Tamai, personal communication).

APPRAISAL AND PERSPECTIVES FOR CLINICAL IMPLEMENTATION

The multitude of approaches to EB treatments and the rapid developments of research methodologies raise our hopes that first evidence-based therapies for EB will enter clinics in the foreseeable future. To date, biologically valid treatment modalities for most severe EB types have advanced to preclinical and clinical testing, but all strategies still face substantial challenges, including technical issues, safety considerations, or issues related to practical clinical implementation and the duration of the clinical effects. Many of the pilot studies have made us realize that much work is still needed for better understanding of the disease mechanisms and skin stem cell properties. These must be further elucidated, and new therapeutic targets identified. Based on all we know today, the prediction is that future treatments for EB will represent individualized medicine based on the patient's mutation constellation, phenotypic characteristics and prominent disease mechanisms. They are likely to encompass combinations of different therapeutic principles: curative and symptom-relief therapies. It is easy to imagine therapeutic regimens using alternating gene, cell and drug therapies to win the best clinical outcomes and to reduce adverse effects. Once therapies are available for wide clinical implementation, the next big challenges will have to be tackled, such as cost and worldwide access to therapy.

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REVIEW ARTICLE

Collagen XVII Processing and Blistering Skin Diseases

Wataru NISHIE
Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Collagen XVII (COL17) is a hemidesmosomal transmembrane protein in the skin, which, in several autoimmune blistering skin diseases, may be targeted by autoantibodies. In addition, loss-of-function mutations in the *COL17A1* gene induce a subtype of junctional epidermolysis bullosa. The extracellular domain of COL17 can be physiologically cleaved from the cell surface by ADAM family proteins in a process known as ectodomain shedding. COL17 ectodomain shedding is thought to be associated with the migration and proliferation of keratinocytes. Furthermore, the C-terminal cleavage of COL17 may be associated with basement membrane formation. COL17 can be targeted by various proteases, including MMP9, neutrophil elastase, plasmin and granzyme B, which may be associated with blister formation in pemphigoid diseases. Interestingly, cleavage of COL17 may induce neoepitopes on the proteolysed fragments, and such induction is associated with dynamic structural changes. This review summarizes the current understanding of cleavage of COL17, and how such cleavage relates to blistering skin diseases.

Key words: ectodomain shedding; BP180; bullous pemphigoid; linear IgA bullous disease; epidermolysis bullosa.

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Corr: Wataru Nishie, Department of Dermatology, Hokkaido University Graduate School of Medicine, N15W7, Kita-Ku, Sapporo 060-8638, Japan. E-mail: nishie@med.hokudai.ac.jp

SIGNIFICANCE

Collagen XVII (COL17, also known as BP180) is an important molecule, which maintains stable adhesion between the dermis and epidermis. Genetic and acquired dysfunctions of COL17 lead to blistering skin diseases. However, the expression of COL17 is tightly regulated, depending on various settings, including wound-healing, proliferation and differentiation. Dysregulation of COL17 processing may be associated with the development of blistering skin diseases; thus, it is important to understand the mechanism by which COL17 is processed and the diseases associated with such processing.

Type XVII collagen (COL17), also known as BP180/BPAG2, is a type-II-oriented transmembrane collagen composed of 3 identical 180-kDa α-chains (1). COL17 is one of the hemidesmosomal components of basal keratinocytes. It links keratin intermediate filaments to the underlying dermis via plectin, BP230, laminin 332 and type VII collagen (2). Loss-of-function mutations in the *COL17A1* gene result in a subtype of junctional epidermolysis bullosa (JEB), which clinically manifests as blister formation and abnormalities of the hair and teeth (3). Since JEB associated with *COL17A1* gene mutations shows a relatively mild phenotype, the disease was previously called "generalized atrophic benign epidermolysis bullosa (GABEB)".

Autoimmunity to COL17 induces bullous pemphigoid (BP), a major autoimmune blistering skin disease, which commonly develops in elderly people (4, 5). In BP, itchy urticarial erythema and tense blisters develop on the entire body, and the mucous membranes may be involved. Major epitopes for BP autoantibodies cluster tightly within the juxtamembranous extracellular noncollagenous 16th A (NC16A) domain of COL17 (6), and previous studies have revealed the pathogenicity of immunoglobulin G (IgG)-class autoantibodies directing this region (7, 8). COL17 may also be targeted by autoantibodies in other autoimmune blistering skin diseases, including mucous membrane pemphigoid (MMP) and linear IgA bullous disorder (LABD) (4).

The two COL17-associated blistering disorders, JEB (GABEB) and BP, suggest that COL17 is a functionally important structural molecule that maintains stable adhesion between the dermis and the epidermis at the dermal-epidermal junction (DEJ). However, basal keratinocytes are dynamic, and they migrate or differentiate in a context-dependent manner. Therefore, processing of COL17 may be involved in various physiological settings. In addition, dysregulated processing of COL17 may be associated with blistering skin diseases. This review summarizes the current understanding of COL17 processing and the blistering skin diseases associated with such processing.

COL17 PROCESSING IN PHYSIOLOGICAL SETTINGS

COL17 ectodomain is constitutively cleaved within the NC16A domain

In cultured keratinocytes, the 120-kDa extracellular domain of COL17 is constitutively shed from the cell surface and is detectable in soluble form in culture me-

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dium (9, 10). COL17 ectodomain shedding is mediated by ADAM 9, 10 and 17 (11), and mass spectrometry analyses have revealed that the cleavage occurs at different regions within the NC16A domain (**Fig. 1**A) (12, 13). The results are consistent with the nature of ADAM family proteins, which cleave substrate proteins more preferentially, based on the distance from the cell surface rather than on amino acid sequences. The detection of cleavage sites within the NC16A domain of COL17 enables the production of cleavage-site-specific antibodies specifically detecting the cleaved COL17 ectodomains. Unique antibodies have revealed that migrating normal human keratinocytes cleave COL17 ectodomains, which co-localize with laminin 332 (Fig. 1B), (14) and cleaved ectodomain fragments exist in the DEJ of normal human skin (13, 15). Interestingly, the cleavage site(s) of COL17 in pathological settings may differ from that in physiological settings (15). In genetically manipulated mice whose NC16A domain includes amino acid sequences that impair ectodomain shedding, the inhibition of COL17 ectodomain shedding somewhat accelerated re-epithelialization after skin wounding (16). The suppression of re-epithelialization by COL17 ectodomain shedding is associated with dampening of mTOR signalling (17). However, wound healing processes differ greatly between humans and mice, with wounds in mice healing mainly by contraction (18). Therefore, further studies are essential to address the physiological roles of COL17 ectodomain shedding in human skin.

C-terminal cleavage of COL17

The cleaved 120-kDa ectodomain of COL17, also called as linear IgA dermatosis antigen (LAD-1) , may be further processed at the C-terminal region around the NC4 domain, which migrates around 97 kDa (19, 20). The 97-kDa processed COL17 polypeptide is called linear IgA bullous disorder (LABD)-97 (**Fig. 2**A). Although it remains uncertain whether LABD-97 is present in normal human skin, C-terminal processing is expected to be physiologically associated with correct basement membrane formation in skin, as described later in this article.

Cleavage in unfolded COL17

Within the NC16A domain, COL17 has a distinct furin consensus sequence: ‘’RIRR”. Early studies have suggested that ectodomain shedding of COL17 may be induced by this distinct motif; however, the furin consensus motif is not used under physiological settings (10). What is the physiological role of the furin consensus motif in COL17? As illustrated in Fig. 1A, there are potential coiled-coil sequences just before the furin consensus motif, and these sequences initiate the folding of COL17

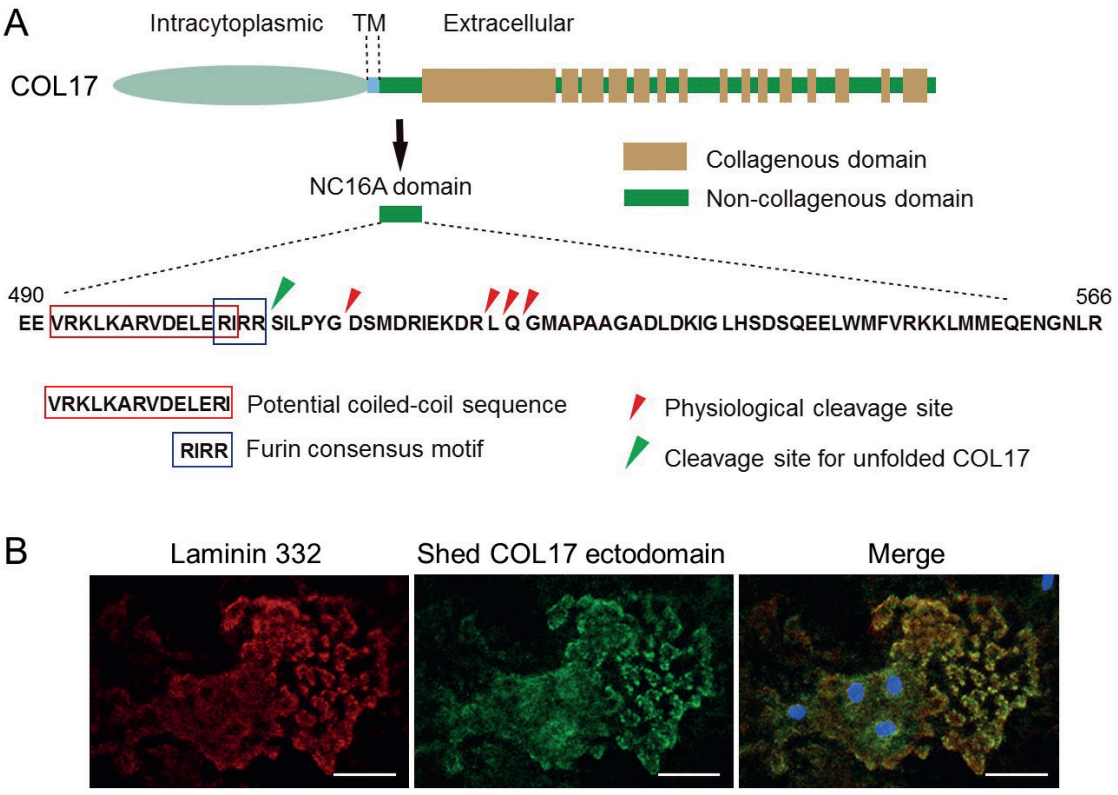

Fig. 1. Collagen XVII (COL17) processing in physiological settings. (A) Schematics of COL17 and sequences of the NC16A domain. (Copyright 2010: The American Association of Immunologists, Inc.). (B) The shed COL17 ectodomain (green: antibody HK139) and laminin 332 (red: antibody 6F12) co-localize in the extracellular matrix of normal human skin. TM: transmembrane. Blue: DAPI. Scale bar: 40 μm. The figures have been partially modified from previous studies (13, 14). Permission given by publisher.

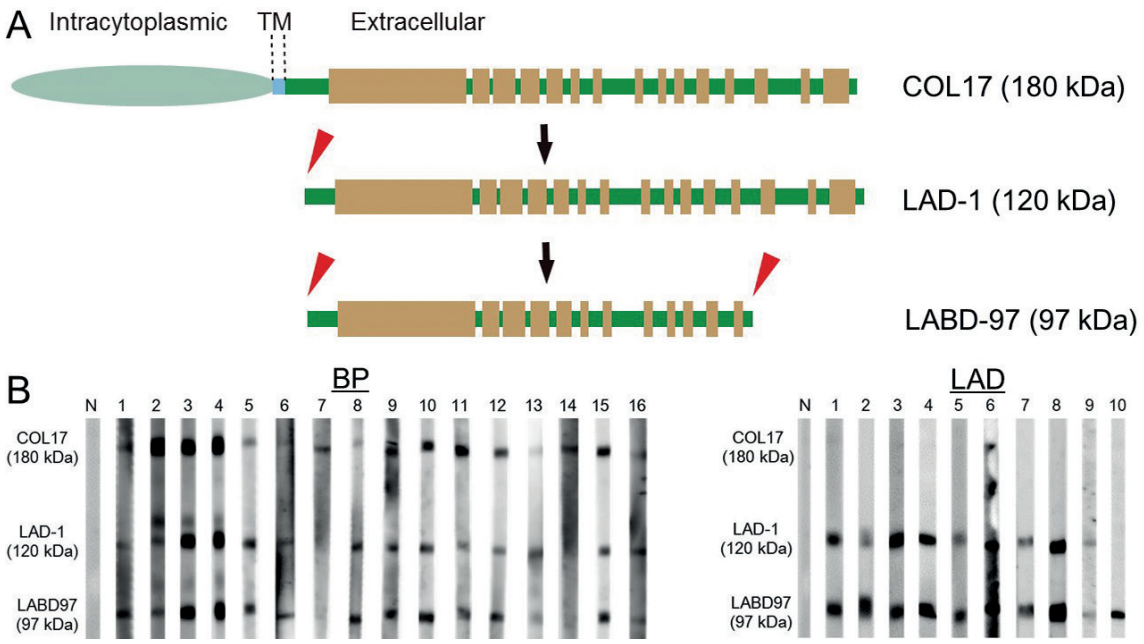

Fig. 2. Neoepitope development in the cleaved collagen XVII (COL17) ectodomains. (A) Schematics of linear IgA dermatosis type 1 (LAD-1) and linear IgA bullous disorder (LABD)-97 polypeptides. (B) LAD IgA-class autoantibodies show more intense reactivity to the cleaved COL17 ectodomains LAD-1 and LABD-97 than to full-length COL17. Note that LAD sera numbers 2, 5 and 10 react more strongly to LABD-97. N: normal control. The immunoblotting data have been partially modified from a previous study (23). BP: bullous pemphigoid. Permission given by publisher.

as a collagen triple helix in the N to C direction (21). When coiled-coil disruptive mutations are introduced, COL17 folding is impaired and unfolded COL17 accumulates in cells. The unfolded COL17 is cleaved by furin at the ''RIRR'' furin consensus motif in the Golgi apparatus before being incorporating into the cell membrane. Finally, cleaved 120-kDa ectodomains derived from unfolded COL17 are expelled from the cells (21). Thus, cleavage at the furin consensus motif within the NC16A domain of COL17 is important for maintaining the quality of the molecule.

COL17 CLEAVAGE AND BLISTERING SKIN DISEASES

Cleavage within the NC16A domain induces neoepitopes in processed COL17 ectodomains

As described, cleavage of COL17 within the NC16A domain yields a 120-kDa ectodomain polypeptide, known as LAD-1 (Fig. 2A). LAD-1 partially contains sequences of the NC16A domain, with which BP autoantibodies preferentially react (12, 13). Similarly, MMP autoantibodies targeting the C-terminal regions of COL17 may react with LAD-1. It is notable that, in some cases of BP and in many cases of LABD, autoantibodies show more preferential reactivity to LAD-1 than to full-length COL17 (Fig. 2B) (19, 22), indicating that cleavage within the NC16A domain induces neoepitopes on the cleaved LAD-1. *In silico* predictions based on detected cleavage sites reveal that the antigenicity of the remnant NC16A sequences in the cleaved COL17 ectodomains increase despite the different cleavage sites (13). Furthermore, monoclonal antibodies target the 15th collagenous (COL15) domain with preferential reactivity to LAD-1, suggesting that cleavage within the NC16A domain induces dynamic structural changes in COL17 (23).

C-terminal cleavage of COL17 induces neoepitopes on the LABD-97 fragment

Since LABD autoantibodies react more preferentially with LAD-1 than with full-length COL17, they usually have strong reactivity to LABD-97 (Fig. 2B) (24). Interestingly, LABD autoantibodies may have greater reactivity to LABD-97 than to LAD-1 (Fig. 2B), suggesting that C-terminal cleavage has additional effects on neoepitope development (23). A previous study reported that epitopes on the 15th collagenous domain appear after C-terminal cleavage (23), which is consistent with an epitope mapping study of LABD autoantibodies (25).

COL17 cleaving enzymes in bullous pemphigoid

In BP lesional skin and blister fluid, several proteolytic enzymes are known to exist, including plasmin, neutrophil elastase and MMP-9 (4, 5). *In vitro* studies have revealed that neutrophil elastase (26), MMP-9 (27) and plasmin (19) are able to cleave COL17. Of these, plasmin is known to cleave within the NC16A and NC4 domains of COL17 ectodomains, yielding 120-kDa LAD-1 and 97-kDa LABD-97 fragments (19, 20, 28). In addition, a recent study has reported that granzyme B, a serine protease secreted by immune cells, is highly expressed

in infiltrated cells in BP lesional skin and that not only does this enzyme cleave COL17, but it also cleaves other molecules present at the DEJ, including α6/β4 integrins and collagen VII (29).

Impaired C-terminal cleavage of COL17 may induce disorganized basement membrane formation

When homozygous R1303Q mutations occur in the *COL17A1* gene, a mild and localized form of JEB develops that is clinically characterized by mechanical blisters, tooth and nail abnormalities, and sclerotic fingers associated with a loss of fingerprints (**Fig. 3**) (30, 31). Histopathologically, duplication of the basement membrane is characteristic of JEB patients with R1303Q mutations (Fig. 3B). Since R1303Q mutations impair the C-terminal processing of COL17, such processing is thought to be essential for normal basement membrane formation in skin (28).

Impaired cleavage of COL17 may induce the breaking of tolerance to bullous pemphigoid autoantigens

BP is induced by autoantibodies targeting the hemidesmosomal components COL17 and/or BP230. Although the pathomechanism of autoantibody-dependent blister formation has been studied extensively, there has been no full elucidation of why tolerance to these autoantigens may be broken in certain individuals. Immune tolerance to molecules may be broken by various triggering events, including thermal burns, ultraviolet (UV) irradiation and surgery (32). In addition, recent studies have reported that anti-type II diabetes mellitus drug dipeptidyl peptidase IV inhibitors (DPP4i) are a risk factor for the onset of BP (33, 34). Furthermore, impaired Treg function may break the tolerance to COL17 and BP230 (35, 36). However, it remains unclear whether the impaired expression of pemphigoid autoantigens may induce the breaking of tolerance. In 2015, Hurskainen et al. (37) produced a genetically manipulated mouse lacking the immunodominant NC14A domain of Col17, a domain that corresponds to the human NC16A domain of COL17. Since NC14A is essential for the ectodomain shedding of mouse Col17, this is another shedding-deficient model. It is notable that the mice are prone to scratching themselves and spontaneously developed anti-Col17 autoantibodies, although no blistering was observed. Whether impairments of BP autoantigens induce the breaking of tolerance had not been elucidated, therefore this study brought important

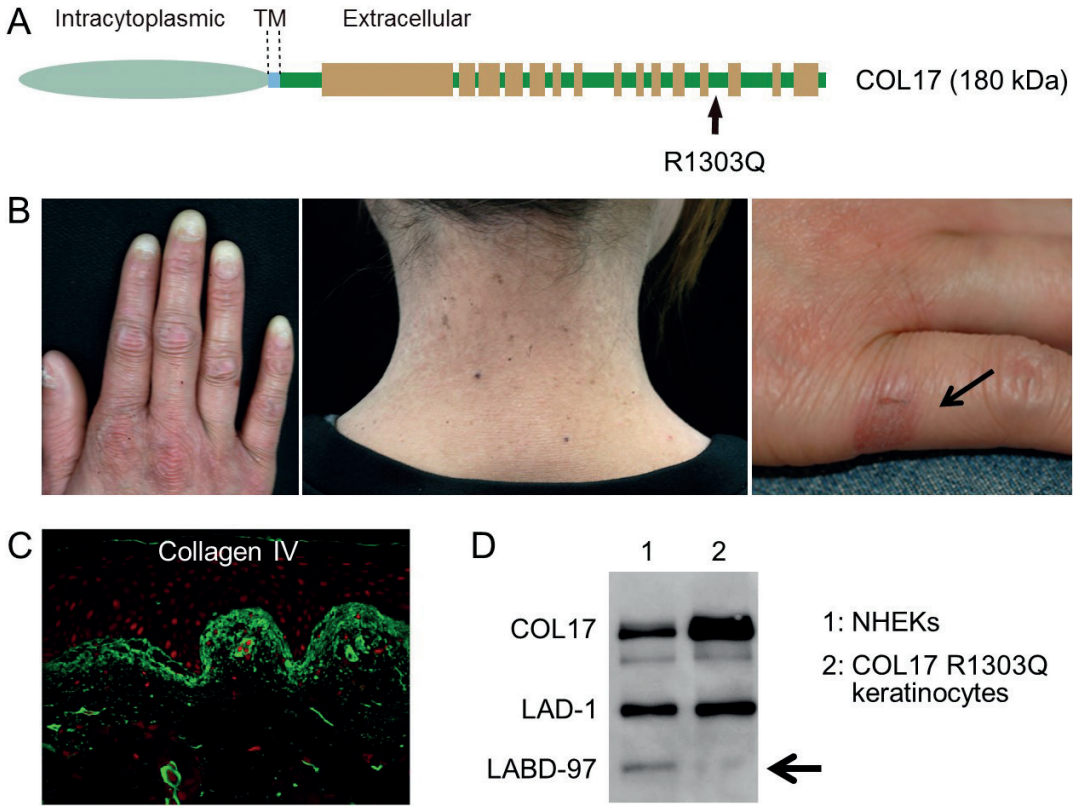

Fig. 3. Collagen XVII (COL17) R1303Q mutation induces blistering disease associated with disorganized basement membrane formation. (A) The R1303Q mutation is located within the NC4 domain. (B) A previously reported 32-year-old COL17 R1303Q$^{+/+}$ patient (28). The *arrow* indicates a mechanical blister. (C) A disorganized and duplicated basement membrane is a characteristic histopathological feature, which can be detected by anti-type IV collagen antibodies (PHM-12+CIV22). (D) Western blotting using anti-COL17 NC16A antibodies (NC16A-3) on extracellular matrix proteins derived from mal human epidermal keratinocytes (NHEKs) and keratinocytes from a R1303Q$^{+/+}$ junctional epidermolysis bullosa patient. The *arrow* indicates that linear IgA bullous disorder (LABD)-97 is absent in R1303Q$^{+/+}$ keratinocytes, suggesting that the C-terminal cleavage of COL17 is impaired. The figures have been partially modified from previous studies (28). LAD-1: linear IgA dermatosis type 1. Permission given by publisher.

information on the pathomechanism behind the breaking of tolerance to COL17.

Cleaved fragments on immune cells in bullous pemphigoid lesional skin

The roles of IgG-class anti-COL17 autoantibodies in the development of blisters have been studied extensively; in contrast, the pathomechanism for urticarial erythema has not been fully elucidated. Previous studies have reported that both IgG- and IgE-class anti-COL17 NC16A autoantibodies are present in BP sera (38, 39). Although *in vivo* IgE deposition at the DEJ may be observed in BP, the positivity rate is not high (40). Notably, Freire et al. recently reported that IgE is rarely observed at the DEJ, but that it is prominent on mast cells and eosinophils in the dermis, in which COL17 ectodomain fragments co-localized with IgE (39). This observation is consistent with the fact that the shed COL17 ectodomain is soluble after being cleaved from the cell surface, as described previously.

CONCLUSION

JEB and pemphigoid diseases have proven that COL17 is a vital player in the stable adhesion between the dermis and epidermis at the DEJ in the skin. However, this adhesion needs to be tightly regulated in a context-dependent manner, for basal keratinocytes to migrate, differentiate and proliferate. Undoubtedly, the processing of COL17 is involved in various normal physiological, as well as pathological, settings, and will be the focus of future study.

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REVIEW ARTICLE

Drug Development in Pemphigoid Diseases

Katja BIEBER and Ralf J. LUDWIG
Lübeck Institute of Experimental Dermatology and Center for Research on Inflammation of the Skin, University of Lübeck, Lübeck, Germany

Pemphigoid diseases are organ-specific autoimmune diseases of the skin and/or mucous membranes. They are caused by autoantibodies targeting adhesion molecules located at the dermal–epidermal junction. While the diagnostics of pemphigoid diseases and insights into their pathogenesis have improved significantly, the development of novel treatments that are effective and safe remains an unmet medical need. However, numerous pre-clinical studies and early clinical trials have recently been launched. This review summarizes some pathways leading to drug development in pemphigoid diseases, namely: (*i*) hypothesis-driven drug development; (*ii*) omics-based drug development; (*iii*) drug repurposing; (*iv*) screening-based drug development; and (*v*) drug development based on careful clinical observations. Ultimately, it is hoped that this will lead to personalized and curative treatments.

Key words: bullous pemphigoid; epidermolysis bullosa acquisita; translational medical research; disease models; animal autoantibodies.

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Corr: Ralf J. Ludwig, Lübeck Institute of Experimental Dermatology University of Lübeck, Ratzeburger Allee 160, DE-23538 Lübeck, Germany. E-mail: ralf.ludwig@uksh.de

SIGNIFICANCE

Despite detailed insights into the pathogenesis of pemphigoid diseases, their treatment still relies on unspecific immunosuppression. Since such treatment contributes significantly to the high patient morbidity and increased mortality, we propose pathways that may facilitate drug development for pemphigoid diseases. With this we aim to foster translational research to develop new treatment strategies for patients with pemphigoid diseases.

(Muco)-cutaneous blistering is the clinical hallmark of pemphigoid diseases (PD). They are characterized and caused by autoantibodies targeting adhesion molecules located at the dermal–epidermal junction. Depending on clinical presentation, the specificity and isotype of the autoantibodies in the following PD can be distinguished (1):

- *Bullous pemphigoid* (BP) is the most prevalent PD and predominantly affects elderly people. BP is caused by autoantibodies targeting BP180 and/or BP230 (2).
- *Mucous membrane pemphigoid* (MMP) is defined as a PD with autoantibodies against components of the dermal–epidermal junction (i.e. BP180 or laminin 332) and predominant mucosal involvement (3, 4).
- *Pemphigoid gestationis* (PG) is a pregnancy-associated immunobullous disease with autoantibodies against BP180 (5).
- *Linear IgA disease* (LAD) is characterized by the linear binding of IgA autoantibodies at the dermal–epidermal junction. LAD is the most common PD in children and clinically presents with urticarial plaques, erosions, and blisters, frequently in a ring-shaped pattern with blistering along the edge of lesions, forming the so-called string-of-pearls sign (6).
- *Epidermolysis bullosa acquisita* (EBA) is a rare and clinically very heterogeneous PD, but due to the availability of pre-clinical model systems it is well-studied (7, 8).
- *Anti-p200/anti-laminin γ1 pemphigoid* (p200) clinically mimics BP, but patients are younger and p200 usually responds well to treatment (9).
- *Lichen planus pemphigoides* (LPP) is, like BP, caused by anti-BP180 antibodies, but in LPP these occur together with lichen planus. Patients with LPP are also younger than those with BP (10).

UNMET MEDICAL NEED IN PEMPHIGOID DISEASES

Treatment of all PD centres on unspecific, systemic immunosuppression, whereby corticosteroids are usually the first line of treatment. Among PD, PG, LAD and p200 usually respond well to treatment and long-term remissions are common. Likewise, BP also responds well to either systemic or topical corticosteroids. However, after withdrawal of treatment, BP relapses in almost 50% of patients within 6 months, requiring long-term corticosteroid treatment, which contributes to patient morbidity and mortality. Both, MMP and EBA are notoriously difficult to treat, and often remission is achieved only after months of immunosuppressive therapy, usually a combination of several drugs (1, 11–16).

This "need for better treatment options" has been identified recently by patients and physicians in a survey to identify the medical need in PD (17). In addition to the current limitations regarding treatment options, the increasing incidence of PD, especially in ageing socie-

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ties (18, 19), further contributes to the medical need to develop novel treatment strategies for PD that are both effective and safe. This increasing medical need has also prompted a significant number of translational studies and clinical trials in PD (20, 21). Unfortunately, however, these clinical trials will not fully address the medical need in PD. Thus, ongoing translational research is required to continuously improve the treatment options, ultimately aiming for personalized and curative treatment.

PATHWAYS TO NEW DRUGS FOR THE TREATMENT OF PEMPHIGOID DISEASES

There are many pathways that may contribute to drug development in PD (**Table I, Fig. 1**). While it may be simplistic, it could be useful to categorize these pathways to new drugs, as follows: (*i*) hypothesis-driven drug development; (*ii*) omics-based drug development; (*iii*) drug repurposing; (*iv*) screening-based drug development; and (*v*) drug development based on careful clinical observations. Examples of each of these pathways to novel treatments for PD are given and discussed in more detail below. The aim of this review is to promote drug development for patients with PD by providing these examples. Another important aim of this article is to initiate a discussion on how this goal is best achieved. Hence, the authors are looking forward to comments from the community, which it is hoped will lead to a fruitful discussion.

Hypothesis-driven drug development: anti-C1s antibodies in bullous pemphigoid

Complement deposition at the dermal–epidermal junction is one of the diagnostic pillars of PD (22). The functional contribution of complement to the pathogenesis of PD has been well documented in pre-clinical model systems (23, 24). Recent data, however, suggests that complement has a more complex role in pemphigoid, whereby some complement receptors confer protection from development of clinical disease (25), or where PD develops independent of complement activation (26). Nonetheless, the complement component C5a has to be considered as one of the main drivers of autoantibody-induced tissue damage in PD (27, 28).

Based on these considerations, function-blocking antibodies to C1s, which initiate the classical complement activation cascade, were developed (29). These anti-C1s antibodies, dose-dependently inhibited the immune complex-induced complement fixation on human skin cryosections (30). More recently, a phase I clinical trial in patients with BP was successfully completed, in which the anti-C1s antibody TNT009/BIVV009 was found to be safe and tolerable in this elderly population, with only mild to moderate adverse events (31). Furthermore, a phase II clinical trial using the dual C5/LTB4 inhibitor coversin is currently being conducted in BP, with promising initial data (32). What is perhaps most striking about the clinical development of these 2 complement inhibitors is the long time needed to translate the clinical and experimental findings on the importance of the complement system into clinical trials. The presence of complement deposits in BP was discovered in the late 60th of the last century (33), and the central role of the complement system in disease pathogenesis was described over 20 years ago (34).

Interestingly, complement activation in PD seems to be restricted to the skin, where C3 deposits are regularly observed, both in patients and animal models of the diseases. More specifically, plasma concentrations of C3a, C4a and C5a in patients with BP were identical to those observed in age- and sex-matched controls. In the same cohort of patients, concentrations of these complement compounds did not change after clearance of skin lesions. In contrast, all of the patients had C3 deposits in the skin at the time of diagnosis (30). Recently, targeted complement therapeutics have been developed, which preferentially bind to sites where complement is activated (35, 36). These targeted complement therapeutics are expected to be both more effective and have fewer adverse events compared with non-targeted complement inhibitors.

Omics-based drug development: validation of spleen tyrosine kinase as a target for treatment of pemphigoid disease

With the availability of novel technologies; for example, genetics, proteomics and RNA sequencing, an unbiased exploitation of novel therapeutic targets can

Table I. Examples of drugs evolving from the outlined pathways to drug development in pemphigoid diseases

Pathway to drug development	Target (compound)	Evidence	Development state
Hypothesis-driven	C1s (Sutimlimab)	Pre-clinical, *in vitro* (30) Phase I trial in patients with BP (31)	Phase I clinical trial completed
	C5/LTB4 (Coversin)	Pre-clinical, *in vivo* (67)	Ongoing Phase IIa in BP (68)
Omics-based	SYK (BAY61-3606)	Pre-clinical, *in vivo* (37, 38)	Target validated in PD mouse model
Drug repurposing	Doxycycline	Case report(s) (series) (15)	Phase III clinical trial successfully completed (43)
	DMF (Skilarence)	Pre-clinical, *in vivo* (47)	Phase II clinical trial in preparation (20)
Drug screening	Not disclosed	Pre-clinical, *in vitro* (69)	Pre-clinical
Clinical observations	Autoantibodies	Case report series (59) Pre-clinical, *in vitro* (60)	Pre-clinical (60)

BP: bullous pemphigoid; DMF: dimethyl fumarate; LTB4: leukotriene B4; PD: pemphigoid disease; SYK: spleen tyrosine kinase.

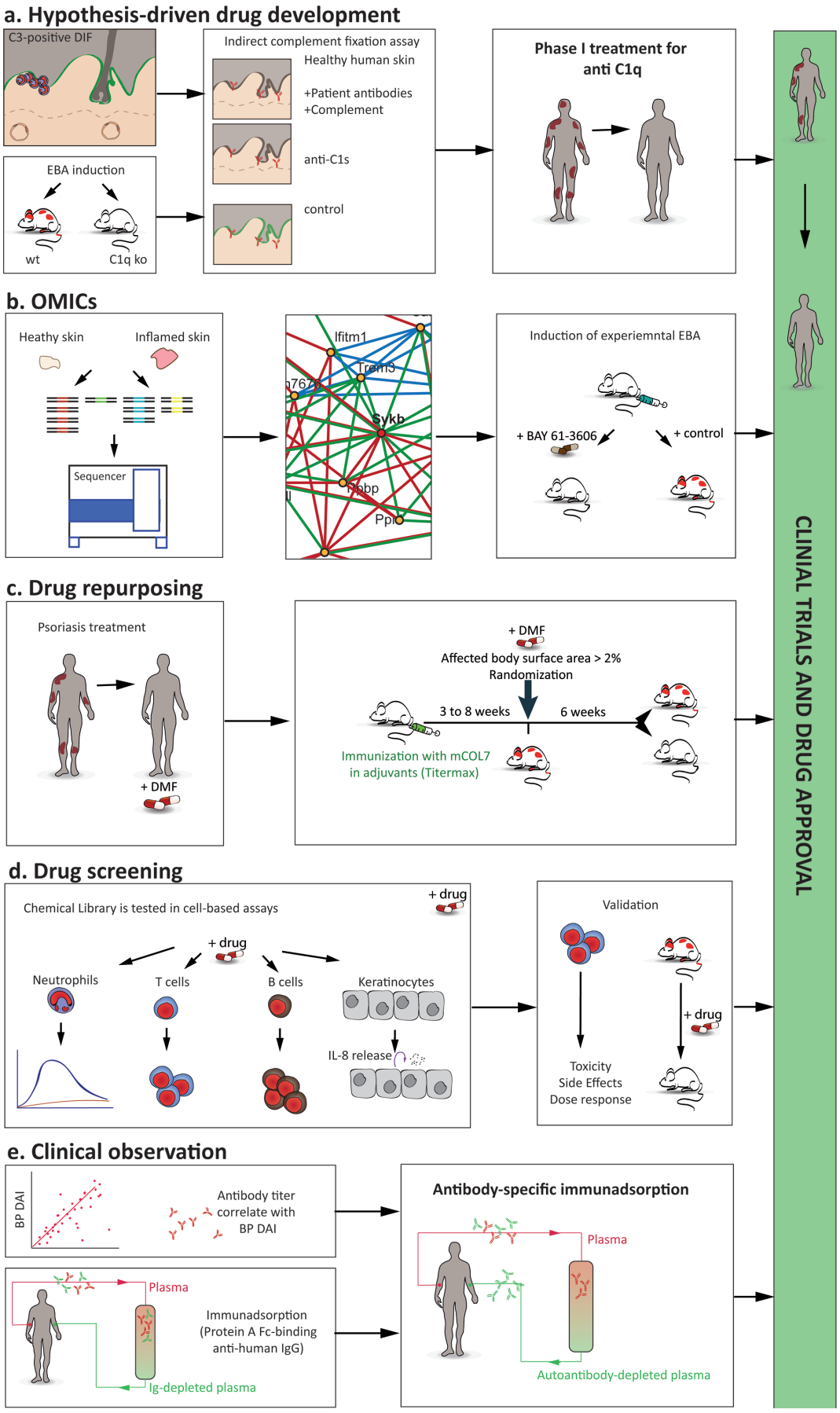
a. Hypothesis-driven drug development
C3-positive DIF
EBA induction
wt
C1q ko
Indirect complement fixation assay
Healthy human skin
+Patient antibodies
+Complement
anti-C1s
control
Phase I treatment for anti C1q
b. OMICs
Heathy skin
Inflamed skin
Sequencer
Ifitm1
Trem3
Sykb
Hpbp
Ppic
Induction of experiemntal EBA
+ BAY 61-3606
+ control
c. Drug repurposing
Psoriasis treatment
+ DMF
+ DMF
Affected body surface area > 2%
Randomization
3 to 8 weeks
6 weeks
Immunization with mCOL7
in adjuvants (Titermax)
d. Drug screening
Chemical Library is tested in cell-based assays
+ drug
+ drug
Neutrophils
T cells
B cells
Keratinocytes
IL-8 release
Validation
+ drug
Toxicity
Side Effects
Dose response
e. Clinical observation
BP DAI
Antibody titer
correlate with
BP DAI
Plasma
Immunadsorption
(Protein A Fc-binding
anti-human IgG)
Ig-depleted plasma
Antibody-specific immunadsorption
Plasma
Autoantibody-depleted plasma
CLINIAL TRIALS AND DRUG APPROVAL

Fig. 1. Pathways to new drugs for the treatment of pemphigoid diseases. (a) Development of new pemphigoid treatments based on hypothesis-driven research. As an example, the development of new complement inhibitors, such as anti-C1s antibodies and coversin are depicted. Based on the clinical observation that complement deposits (in *green*) are highly prevalent in the skin of patients with pemphigoid disease (PD) (1) and the observation that mice deficient (ko) in specific complement proteins are protected from the induction of experimental PD (34), targeting complement activation was assumed to have disease-modifying effects in PD. Both (*in vitro* assays, *middle panel*) and a phase I clinical trial demonstrated that anti-C1s impairs/reduced complement deposition along the dermal-epidermal junction. (b) Development of new pemphigoid treatments based on complex data sets and omics. Here biological specimen, i.e. affected vs. non-affected skin from patients or pre-clinical model systems (*left-hand image*), are subjected to unbiased measurement, for example RNA-sequencing or proteomics. In the example provided, RNA expression in the skin was contrasted between healthy mice and mice with EBA. Subsequently (*middle image*), data analysis is performed, leading to the identification of potential pharmacological targets, such as *Sykb*. For functional validation, in vitro systems or pre-clinical model systems (*right-hand image*) may be used. (Example from Samavedam et al. (37)). (c) Development of new pemphigoid treatments based on drug repurposing. Already licensed drugs, for indications than other pemphigoid, can be repurposed for PD. The rationale for drug repurposing in pemphigoid can either be based on clinical observations, i.e. case report series that a given drug is also effective in pemphigoid, such as doxycycline, or be hypothesis-driven, as shown for dimethyl fumarate, which has a long history as an anti-psoriatic agent (*left-hand panel*), which also ameliorates experimental PD (*right-hand panel*). (d) Development of new pemphigoid treatments based on drug screening. If putative defined drug targets are not known, drug screening can be performed in in vitro model systems, which are up-scalable and highly reproducible. In PD, examples for these assay systems are immune complex-induced release of reactive oxygen species from neutrophils, anti-CD3/CD28-induced T cell proliferation, IL-21/antiCD40L-induced B cell proliferation and anti-BP180 IgG-induced cytokine release from keratinocytes (51). Drug libraries, for example the Prestwick Chemical Library (66) or the FDA-approved Drug Library from Selleckchem, can be obtained commercially. After the initial screening the identified potential drugs need to be validated *in vitro* and *in vivo* (left-hand panel). (e) Development of new pemphigoid treatments based on clinical observations. After the identification of the pathogenic relevance of autoantibodies in PD and the clinical observation of a correlation of the levels of the circulating autoantibody titres with disease severity, immunoadsorption/plasmapheresis were introduced to the management of PD. However, immunoadsorption is limited because all antibodies are removed. Hence, the procedure has to be paused, and does not elute all autoantibodies from the patients. Using the insights from detection of specific autoantibodies in PD, first attempts were made to develop antigen-specific immunoadsorption.

be performed. Regarding PD, such approaches have, however, been sparsely used, and have been limited to mouse models (7). In detail, contrasting cutaneous RNA expression from mice with and without experimental EBA, several potentially disease-promoting genes were identified, i.e. *Sykb*, the gene encoding for the spleen tyrosine kinase (SYK). To evaluate the functional role of differential *Sykb* expression in EBA, experimental EBA was induced in mice that were treated with selective SYK inhibitors, or EBA was induced in SYK-deficient mice. In both experiments, complete protection from induction of experimental EBA was observed if SYK was blocked (37). In parallel, hypothesis-driven research, made similar observations (38). Thus, SYK has been independently identified and validated as a potential therapeutic target for PD.

Unfortunately, however, omics datasets are quite sparse for PD. To the best of our knowledge, only one GWAS has been published so far, reporting an association of MMP with *HLA-DQB1*03:01* and rs17203398, in which the intronic region of *GALC* is located (39). Therefore, in the future, a joint community effort is required to collect well-defined patient samples using standardized procedures for sample acquisition and storage. Alternatively, or in parallel, multi-omics data from model systems (as reported for SYK) may be used for target identification, as well as functional validation. For translation into clinical use, expression of the identified targets may be performed in corresponding patient samples. The advantage of such an approach is that fewer patient samples would be required.

Drug repurposing: doxycycline and dimethyl fumarate for bullous pemphigoid treatment

In dermatology, the use of the anti-CD20 antibody rituximab, initially developed for the treatment of B cell malignancies (40), for the treatment of pemphigus (41) is a good example of drug repurposing. In contrast to "conventional" drug development, already licensed compounds are evaluated for efficacy in other indications. The already known safety profile of the licensed drugs, the decreased time and costs of drug approval are the main advantages of drug repurposing (42).

Regarding PD, the antibiotic doxycycline has recently been demonstrated to be effective in the treatment of BP (43). In a comparative clinical trial, 200 mg of doxycycline, achieved clinical remission in 74% of patients within 6 weeks; while prednisolone (initial dose 0.5 mg/kg) induced remission in 91% of patients. Regarding adverse events, 18% of doxycycline-treated patients experienced a grade 3 or greater adverse event. This was significantly lower, compared with prednisolone, where the number of adverse events was 2-fold higher. Another compound that is currently evaluated for repurposing in BP is dimethyl fumarate (DMF). In Germany, the compound has a long-standing history as an anti-psoriatic agent (44), and more recently has also been licensed for treatment of multiple sclerosis (45). DMF has a multitude of biological effects, including a shift in cytokine expression, a suppression of leukocyte extravasation, anti-oxidant properties, and many others (46). Based on these properties, we hypothesized that DMF may also be beneficial for the treatment of PD. Indeed, treatment of mice with already established clinical EBA manifestations led to a significant improvement in disease activity, while clinical disease severity increased in solvent-treated mice (47). On a molecular level, the beneficial effects of DMF in EBA are mediated through the hydroxycarboxylic acid receptor 2 (48). Based on these findings, the DPem consortium was established to evaluate the safety and efficacy of adjuvant DMF in BP patients responsive to corticosteroid treatment. Centres in France, Poland, Turkey and Germany will recruit 210 patients with BP and allocate these to DMF or placebo.

To the authors' knowledge there are additional drugs soon to be published that have the potential for repurposing in PD. We expect that this pathway to novel drugs for PD will lead to the approval of several new treatment options for pemphigoid patients, using "old" drugs from other indications.

Drug screening

The use of chemical libraries to identify inhibitors of specific molecules, or the use of complex, but up-scalable, model systems is well established for drug development (49, 50). While the use of specific (enzymatic) assays is very well suited to identify new compounds for known pharmacological targets, the use of complex, up-scalable systems in chemical screens offers advantages in instances where molecular defined targets are not known. Despite the fact that up-scalable complex *in vitro* models of PD are already established (51), these have, so far, not been used for drug development in pemphigoid. Examples of these up-scalable model systems are immune complex-induced release of reactive oxygen species (ROS) from neutrophils, or autoantibody-induced cytokine release from keratinocytes, as well as stimulation of T cells using anti-CD3/CD28 and B cell stimulation with IL-21 and anti-CD40L (51, 52).

An envisioned work-flow of such an approach would be to screen compounds of a chemical library to inhibit activation of immune cells or autoantibody-induced cytokine release from keratinocytes with a relatively small sample size. Candidate compounds would be selected based on pre-defined cut-off criteria. Subsequently *in vitro* and *in vivo* validation (using appropriate animal pre-clinical model systems (53), would be employed before clinical trials.

It is hoped that these models, as well as computational approaches to drug development, such as the Connectivity Map (54), will lead to the identification of novel compounds suited for the treatment of PD.

Clinical observations: immunoadsorption for bullous pemphigoid

The detection of IgG deposits along the dermal–epidermal junction in PD (55) and the identification and cloning of the corresponding autoantigens (56) led to the development of serological test systems for the diagnosis of PD (1). This, by itself, is a good example, of how clinical observations and basic research can improve diagnosis. In addition, insights into the pathogenetic role of these autoantibodies (24) prompted the use of immunoadsorption/plasmapheresis in PD (57). More recently, 2 case series have been published, reporting the outcome of immunoadsorption in 26 patients with BP. Interestingly, and in contrast to other autoimmune skin blistering diseases, such as pemphigus, long-lasting remissions were observed in the majority of patients (58, 59). This data, however, should be interpreted within the limitations of case series, as well as the use of concomitant treatments.

Currently, removal of autoantibodies by immunoadsorption is, however, limited because all antibodies are removed, rather than selective removal of autoantibodies. Hence, vigorous and prolonged removal cannot be performed using unspecific immunoadsorption. In mice, at least, this limitation has been overcome: by using insights on the autoantigens in pemphigus and PD, which are currently exclusively used for diagnosis (22), columns specifically removing autoantibodies targeting the NC16A domain and Dsg3 were developed, and (in part) successfully employed in animal models (60, 61). If these insights from pre-clinical model systems can be translated into clinical use, immunoadsorption will most likely become a more widely used treatment modality for PD. Another, potentially very selective and antigen-based, treatment is the use of chimeric autoantigen receptor (CAAR) T cells, which have been shown to selectively deplete specific autoreactive B cells in mouse models of pemphigus (62).

FUTURE DIRECTION OF TRANSLATIONAL RESEARCH IN PEMPHIGOID DISEASES

With the increasing number of clinical trials in PD (21), approval of several new treatments for PD can be expected within the next 3–5 years. However, these trials only recruit patients with BP. For all other PD, to the best of our knowledge, there are currently no ongoing clinical trials, despite the high medical need in MMP and EBA. Therefore, specific, or maybe basket, trials that also include these patients would be highly warranted. Regarding curative treatments, the above-mentioned approaches towards the development of antigen-specific immunoadsorption for BP, or the CAAR-T-cell approach could be tailored to each patients' autoantibodies. In particular, removing the autoreactive B/plasma cell population could induce long-lasting remission, or even a cure, for PD. While translating these interesting findings from pre-clinical model systems into clinical use will take considerable time, a personalized treatment for PD could be implemented relatively quickly using established diagnostic and therapeutic procedures: in single-centre and retrospective studies, several biomarkers have been identified that indicate relapse in BP; for example, the presence of anti-type VII collagen autoantibodies, variations of the glucocorticoid receptor β, or CXCL10-induced matrix metalloproteinase 9 secretion (63–65). Given, that (some of) these are validated in prospective multicentre diagnostic clinical trials, tapering of immunosuppression could be adjusted to the expression of these biomarkers.

Collectively, the high medical need to develop new treatments for PD has prompted a very exciting new area

of translational research in this field, which is expected to improve the treatment of patients with PD in the future. New drug approvals, more clinical trials, and personalized and curative treatments are expected.

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Centenary theme section: BLISTERING SKIN DISORDERS

REVIEW ARTICLE

Current Concepts of Dermatitis Herpetiformis

Teea SALMI[1,2] and Kaisa HERVONEN[1,2]
[1]Celiac Disease Research Center, Faculty of Medicine and Health Technology, Tampere University, and [2]Department of Dermatology, Tampere University Hospital, Tampere, Finland

Dermatitis herpetiformis (DH) is an autoimmune skin disease that causes itchy, blistering rash, typically on the elbows, knees and buttocks. DH and coeliac disease share the same genetic background, gluten-dependent enteropathy and antibody response against tissue transglutaminase. DH is currently considered a cutaneous manifestation of coeliac disease, and the prevailing hypothesis is that DH develops as a late manifestation of subclinical coeliac disease. The incidence of DH is decreasing contemporarily with the increasing incidence of coeliac disease. The IgA immune response in DH skin is directed against epidermal transglutaminase, while the autoantigen in the gut is tissue transglutaminase. Granular IgA deposition in the papillary dermis is pathognomonic for DH, and is a finding used to confirm the diagnosis. The treatment of choice for DH is a life-long gluten-free diet, which resolves the rash and enteropathy, increases quality of life, and offers a good long-term prognosis.

Key words: dermatitis herpetiformis; coeliac disease; gluten-free diet; transglutaminase; immunoglobulin A; villous atrophy.

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Corr: Teea Salmi, Department of Dermatology, Tampere University Hospital, PO Box 2000, FIN-33521 Tampere, Finland. E-mail: teea.salmi@tuni.fi

SIGNIFICANCE

Dermatitis herpetiformis is an itchy, blistering rash, which occurs on the elbows, knees and buttocks. Dermatitis herpetiformis is considered a cutaneous manifestation of coeliac disease. Even though obvious gastrointestinal symptoms are rare in dermatitis herpetiformis, intestinal coeliac-type villous atrophy or inflammation is present at diagnosis. The diagnosis is confirmed by skin biopsy revealing typical IgA deposits, and the majority of patients also have coeliac autoantibodies in the serum. The treatment of choice for dermatitis herpetiformis is a life-long gluten-free diet, which resolves the rash and enteropathy, increases quality of life, and offers a good long-term prognosis.

Dermatitis herpetiformis (DH) is an intensively itching skin disease, which causes papulovesicular eruption, predominantly on the elbows, knees and buttocks. DH is considered an autoimmune-based disease, since pathognomonic granular immunoglobulin A (IgA) response in the dermis, directed against epidermal transglutaminase (TG3), and circulating autoantibodies against tissue transglutaminase (TG2) and TG3 exist in DH (1, 2). Moreover, the predisposing genetic background, more specifically HLA DQ2 or DQ8 haplotypes, is a necessity for development of the disease (3). DH is considered a specific variant of coeliac disease, manifesting primarily in the skin, but coeliac-type enteropathy also exists in DH, albeit more subtle than in coeliac disease (4). Currently approximately 13% of patients with coeliac disease have DH (5, 6) and the highest reported prevalence of DH to date has been 75 per 100,000 from Finland (5). The prevalence is lower in some areas of the globe and in specific populations, for example in Asia and in African-Americans (7, 8) and, overall, the geographical differences in the prevalence of DH and, likewise, coeliac disease, have been explained mainly by HLA genetics and wheat consumption habits (9). Also the incidence figures of DH have ranged from 0.4 to 3.5/100 000/year, even in different studies performed in Europe or North America (5, 10). DH is typically diagnosed during adulthood, and the incidence of DH is highest in females and males aged 50–69 years (5, 6). Interestingly, the diagnostic age of DH has increased (5) and, although the reasons for this increase remain largely obscure, a possible explanation could be changes in dietary habits. Nonetheless, even though childhood diagnosis is rare in northern Europe (5, 6, 11) it seems to be more common in Italy and Hungary (12, 13).

The focus of this review is to describe the current, clinically relevant, concepts of DH diagnostics, treatment and prognosis. In addition, the close link between DH and coeliac disease is elaborated, and unique features of DH, the cutaneous manifestation of coeliac disease, are presented.

SKIN MANIFESTATION OF COELIAC DISEASE

The clinical manifestations of DH were first described as early as 1884 by Louis Duhring (14) and, 4 years later, the classical abdominal and malabsorptive symptoms of coeliac disease were described by Samuel Gee (15). The link between DH and coeliac disease was found when Marks et al. (16) detected that coeliac-type enteropathy was also a common finding in DH, and importantly, when gluten-free diet (GFD), the treatment of choice in coeliac disease, was shown to heal small bowel mucosal

changes in DH, and to alleviate DH rash (17, 18). Subsequent family and genetic studies have coupled DH and coeliac disease even more convincingly together: DH and coeliac disease have been shown to occur often in the same families and even in monozygotic twins, and furthermore, predominantly HLA DQ2 and, more rarely, DQ8 haplotypes have been shown to be the predisposing haplotypes in both (19–21). Moreover, it has been shown that the phenotype of coeliac disease is not invariably constant, since it can convert from classical disease into DH, especially when dietary compliance is poor (22).

A major breakthrough occurred in coeliac disease research in the 1990s when TG2 enzyme was identified as the autoantigen of the disease (23). Subsequently an enzyme-linked immunosorbent assay (ELISA)-based method for detecting TG2 antibodies was developed and found to be accurate in coeliac disease (24) and, furthermore, a similar TG2 antibody reaction was shown to occur in the serum of patients with DH (2). Moreover, TG2-targeted autoimmune response has been detected in the small bowel mucosa of untreated coeliac disease and DH patients (25, 26).

DH, however, has some distinct features compared with coeliac disease in general. DH is more rarely diagnosed during childhood compared with coeliac disease (11, 27). Furthermore, DH is slightly more common among males than females (5), which contradicts the female predominance known to exist in coeliac disease (6, 28). Moreover, the incidence of DH has decreased, but in coeliac disease a marked increase in the incidence figures has been detected (5, 6, 28). One prevailing hypothesis is that DH develops as a late manifestation of coeliac disease, affecting individuals with subclinical or neglected coeliac disease. It has, moreover, been suggested that the TG3 immune response typical for DH develops as an epitope spreading phenomenon from an autoimmune response initially targeting TG2 (29). Coeliac-type dental enamel defects detected in adults diagnosed with DH indicate that these individuals were already sensitive to gluten in early childhood (30). Moreover, the rarity of childhood DH and the changing phenotype of coeliac disease during poor dietary adherence support this hypothesis, and furthermore, the divergent trend of incidences of DH and coeliac disease also fits well with this hypothesis: better diagnostics of coeliac disease due to increased awareness, availability of accurate serum autoantibody tests, and screening of risk groups has resulted in a smaller pool of patients with undiagnosed coeliac disease and, consequently, fewer individuals with potential for development of DH.

DIAGNOSING DERMATITIS HERPETIFORMIS

The suspicion of DH typically arises from the characteristic skin symptoms, which are an intensely pruritic rash with small blisters and papules affecting most commonly the extensor surfaces of the elbows, knees and buttocks (**Fig. 1**a, b and **Table** I). Occasionally other sites, such as the scalp, face, upper back and neck, are also affected. There is individual variation in the severity of the rash and pruritus, but commonly due to the intense itch and scratching, the blisters are broken and only erosions, crusts and post-inflammatory hyperpigmentation are consequently present. Acral purpura is one, albeit quite rare, finding in DH and can be found either as a sole presentation or concomitantly with the typical DH rash (31–33). Despite the gluten-sensitive enteropathy, obvious gastrointestinal symptoms and signs of malabsorption are rare in DH, but some kind of abdominal symptoms have, however, been reported in up to one-third of patients (34, 35). Interestingly, although the clinical picture of coeliac disease has been shown to become milder and more heterogenic with increasingly common non-classical symptoms (36–38), it seems that the clinical picture and the severity of DH rash have remained quite unchanged during recent decades (39).

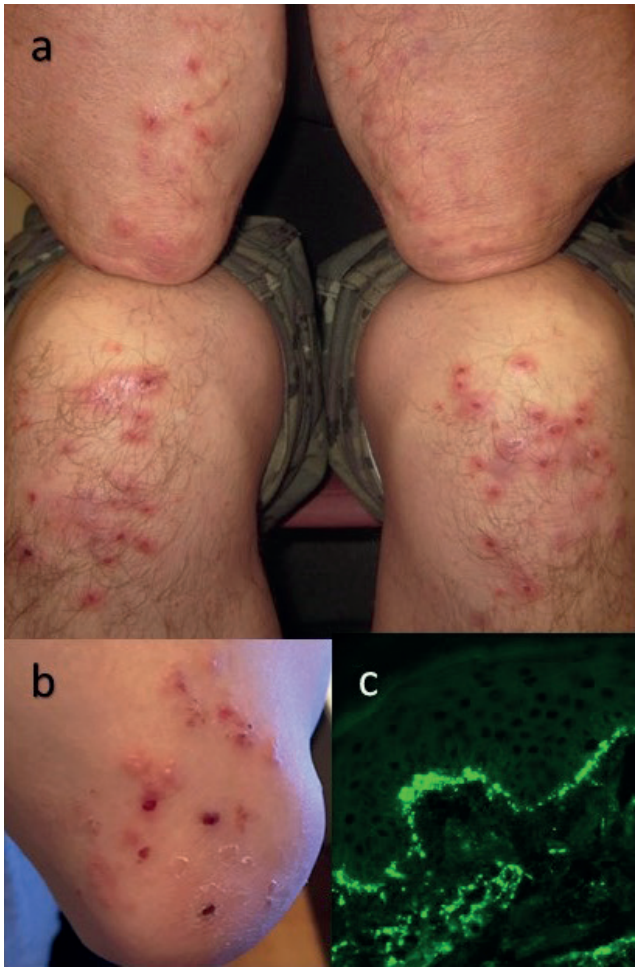

Fig. 1. Clinical characteristics of dermatitits herpetiformis (DH). (a) A typical clinical picture of dermatitis herpetiformis (DH) with excoriated blisters and papules on the elbows and knees. (b) Intact and excoriated blisters, papules and crusts on the elbow. (c) Direct immunofluorescence (×40) finding in DH; granular IgA deposits in the basal membrane zone and in the dermis.

Table I. Diagnostic procedures in dermatitis herpetiformis (DH) and recommendations regarding when they should be applied

Procedure	Recommendation
Patient history and physical examination	
Duration, severity and type of skin symptoms	Always
Presence of gastrointestinal and malabsorptive symptoms and signs	Always
Family history of coeliac disease and DH	Always
Presence of associated autoimmune diseases	Always
Diagnostic procedures	
Direct immunofluorescence examination of perilesional skin biopsy	Always
Histopathological analysis of lesional skin biopsy	In obscure cases
Serum tissue transglutaminase or endomysial antibodies	Always
Small bowel biopsy examination	Only if gastrointestinal symptoms not compatible with coeliac disease exist
HLA DQ2 and DQ8 typing	Only in obscure cases

The differential diagnosis of DH includes other subepidermal blistering diseases, especially linear IgA disease and bullous pemphigoid. In addition, other itchy skin diseases, such as atopic and nummular dermatitis, lichen planus, urticaria and scabies may sometimes be difficult to differentiate from scratched DH rash, although the typical predilection sites of these diseases differ from those of DH (40).

The gold-standard method to verify DH diagnosis is direct immunofluorescence (IF) examination, which shows the pathognomonic granular IgA deposits in the papillary dermis and/or at the dermoepidermal junction (Fig. 1c). IgA deposits are widespread, but not totally uniformly distributed in the skin of patients with DH, and therefore the ideal site for the diagnostic skin biopsy is uninvolved perilesional skin, where the deposits are found in greater amounts (41). The immune response in DH skin is directed against TG3, an enzyme closely related, but not identical, to TG2 (1). It has recently been demonstrated that TG3 disappears from the dermis of patients with DH on a GFD, in parallel with IgA, but the disappearance is prolonged, often taking years even on a strict diet (42). There are a few rather interesting studies reporting that granular IgA also exists in the skin of coeliac disease patients with healthy skin or with inflammatory skin diseases other than DH (43, 44). However, the number of the patients in these studies has been small, and further research evidence is needed before conclusions can be drawn about the existence of granular IgA in non-DH skin. For the time being, at least, this finding can be considered DH-specific.

In addition to the characteristic granular deposition of IgA, mostly sporadic cases of fibrillary IgA deposits in DH have been presented (45–47). The fibrillar pattern of IgA appears to be more common in Japan, where it has been reported to occur in approximately one-third of patients with DH. However, Japanese patients with DH also show other distinct features that differ from Caucasian patients; the Japanese patients with DH do not carry the predisposing HLA-DQ2 and HLA-DQ8 haplotypes, the occurrence of gluten-sensitive enteropathy is rare, and coeliac-disease-specific autoantibodies are seen only in low proportion of patients. These findings suggest that the pathogenesis of Japanese DH differs from that of Caucasian DH, and may not be dependent on gluten (48, 49).

Histopathological examination of lesional skin biopsy is not required for diagnosis of DH, but, in obscure cases, compatible findings with DH support the diagnosis (40). Ideal areas for histopathological biopsy specimen are an intact vesicle or erythematous skin, and the typical findings include non-specific subepidermal blister and papillary microabscesses, together with neutrophil and a few eosinophil infiltrates (50). However, the above-mentioned findings alone do not allow the differentiation of DH from other autoimmune bullous disorders.

A recent study from Finland demonstrates that diagnosis of DH is not always easy. The study investigating the diagnostic delay of DH during the last 45 years detected that the duration of skin symptoms before the diagnosis was 2 years or more in one-third of patients with DH. Female sex, villous atrophy at diagnosis, and a DH diagnosis prior to the year 2000 were significantly associated with long diagnostic delay. Fortunately, the same study established that the diagnostic delay has shortened during recent decades from 12 to 8 months (39). Correspondingly, the diagnostic delay in coeliac disease has become shorter (51).

SEROLOGICAL AND SMALL BOWEL MUCOSAL FINDINGS IN DERMATITIS HERPETIFORMIS

In DH, there are often circulating IgA-class autoantibodies against both transglutaminase isoenzymes, TG2 and TG3. TG2 is also the target for endomysial antibodies (EmA) (52), and ELISA-based TG2- and indirect IF-based EmA tests can equally be utilized in clinical practice (Table I). However, the evaluation of EmA is subjective and requires skilful laboratory personnel. TG2 antibodies have proven to be highly accurate in coeliac disease, but in DH these antibodies are mostly confined to those patients with small bowel mucosal villous atrophy, and hence a negative result does not exclude DH (53). However, together with a compatible clinical picture, TG2 antibodies are suggestive of DH, and further, indicative of small bowel mucosal damage. If elevated, TG2 antibody measurement can further be utilized in the follow-up of GFD adherence after the diagnosis. Circulating TG3

antibodies have been suggested to be DH-specific, but surprisingly, these antibodies occasionally also occur in the serum of coeliac disease patients without any detectable skin lesions (1, 54, 55). It has been shown, however, that in coeliac disease the affinity of the antibodies to TG3 is lower than in DH (1) and that TG3 reactivity increases with age in coeliac disease (55). Therefore, it can be speculated that skin symptom-free coeliac disease patients with TG3 reactivity are susceptible to future development of DH, especially if not compliant with a strict GFD. However, since the exact role and value of TG3 antibodies in DH and coeliac disease is, thus far, to some extent obscure, these antibodies are currently mostly used in research settings.

Small bowel mucosal biopsies obtained during upper gastrointestinal endoscopy are not necessary for DH diagnosis. It is widely recognized that the majority of the untreated DH patients have coeliac-type small bowel mucosal villous atrophy, but at least one-quarter of the patients evince normal villous architecture (53). However, virtually all subjects without evident small bowel mucosal damage evince intestinal coeliac-type inflammation and/or immune response. Characteristic for both DH and coeliac disease is increased densities of γδ+ intraepithelial lymphocytes in the small bowel mucosa (56), but even more specific finding is the presence of intestinal TG2-targeted autoantibody deposits (25, 26). However, both of these investigations require frozen small bowel mucosal samples, which are not available in every diagnostic centre. Importantly, even though small bowel mucosal changes vary from inflammatory changes to severe villous atrophy in DH, recent evidence has shown that the severity of mucosal damage at diagnosis does not have any effects on the long-term prognosis of DH (57, 58), which naturally strengthens the rationale behind the current policy of not obtaining routine small bowel biopsies when DH is diagnosed.

GLUTEN-FREE DIET AND DAPSONE TREATMENT IN DERMATITIS HERPETIFORMIS

The essential treatment for DH is a strict, life-long GFD. When adhering to a GFD, wheat, rye, barley and foods otherwise containing gluten are permanently excluded from the daily diet, but gluten-free oats (i.e. oats not contaminated by other cereals) are currently allowed in most countries and tolerated by the majority of patients with DH (59). Adherence to a GFD leads to healing of the small bowel mucosa and alleviation of the clinical symptoms, but total clearance of the DH rash may take several months or even a couple of years (17, 60). Therefore, at the beginning of GFD treatment the individuals with widespread, active rash need additional treatment with dapsone.

Dapsone is a sulfone drug with potent antimicrobial and anti-inflammatory properties, which relieves the DH rash and itch effectively, but has no effect on the enteropathy. The starting dose of dapsone should be 25–50 mg/day. If needed, the dose can be increased gradually up to 100 mg/day, and then, once the rash has disappeared, the dose should be slowly tapered and finally discontinued as the GFD alone controls the rash (60). Dapsone is usually well tolerated when recommended doses are used, but side-effects are possible, of which dose-dependent haemolysis is the most common and, for example, methaemoglobinaemia, agranulocytosis and hepatitis less frequent. Hence, clinical and laboratory monitoring during treatment is necessary. In Finland approximately 70% of patients with DH require dapsone treatment after being diagnosed, and when initiated, it is usually needed for 2–3 years (57, 60). In rare cases of DH, the rash continues despite long-lasting, strict, adherence to a GFD. Recently this condition, named refractory DH, was found to occur in less than 2% of patients with DH (61). The patients with refractory DH in that study had followed a strict GFD for a mean of 16 years, but dapsone was still essential due to the active DH rash. Interestingly, despite the ongoing clinical symptoms, the small bowel mucosa had recovered in all subjects, and none had developed lymphoma, which suggests that refractory DH probably diverges from refractory coeliac disease, in which the small bowel mucosa does not heal on a GFD and the risk of lymphoma is increased (62). However, since refractory DH seems to be very rare, in cases of non-responsive DH, intentional or accidental dietary lapses are a more common reason and have to be excluded by dietary consultation.

Current recommendations are that treatment with a GFD should be life-long in DH, as in coeliac disease. However, there are some reports suggesting that a proportion of patients with DH following a GFD could later re-introduce gluten to their diet without developing symptoms or signs of DH (60, 63, 64). Three gluten-challenge studies have also investigated the possible redevelopment of gluten tolerance in DH. The first gluten-challenge study by Leonard et al. reported 11 out of 12 (92%) patients with DH relapsed with rash and 7 (64%) of these also with villous atrophy (65). However, when Bardella et al. later challenged 38 GFD-treated DH patients with gluten, they reported 7 (18%) who did not manifest any type of relapse in the skin or small bowel during the prolonged gluten challenge (66). Very recently, a 12-month gluten-challenge study was performed in 19 long-term GFD-treated DH patients in Finland (67). In this study, 18 (95%) of the patients relapsed in a mean of 6 months; 15 (79%) developed DH rash, 12 of whom also showed small bowel villous atrophy, and 3 patients showed progression of small bowel mucosal villous atrophy without skin symptoms or cutaneous IgA deposits. One patient, however, did not show any skin symptoms or IgA deposits, nor did he develop intestinal villous atrophy or inflammation. However, a long follow-up is needed

before it can be concluded that gluten is truly tolerated by this patient, and at present, it seems that development of gluten tolerance in DH is rare or even non-existent, and life-long strict adherence to a GFD is still justified in all patients with DH.

Long-term prognosis on a gluten-free diet

Coeliac disease is known for increased all-cause and lymphoma mortality risk (68). Therefore it is interesting that, in a recent Finnish DH study, the all-cause mortality rate in DH was, in contrast, significantly decreased (standardized mortality rate 0.70), and the lymphoma mortality was increased during the first 5 years after diagnosis, but not thereafter (58). Similarly, a previous DH study from the UK found a slightly, but non-significantly, reduced mortality rate (hazard ratio 0.93) (69). In the Finnish study, 98% of patients with DH adhered to a GFD, which may explain their excellent prognosis, whereas in the study from the UK, data about dietary adherence was absent for one-third of patients (58, 69). Evidence clearly confirms that adherence to a GFD reduces the risk of lymphoma in DH, the risk of which has been shown to be similarly increased in DH and coeliac disease (70–72). In DH, the risk of gastrointestinal carcinomas has not been reported to be increased, which is in contrast to coeliac disease (69, 71, 72). Also, the increased bone fracture risk associated with coeliac disease seems not to be a complication of similar extent in DH, although bone complications have been very rarely studied in DH (69, 73).

Quality of life (QoL) aspects in coeliac disease have been widely studied, but only limited evidence of DH and QoL exist. However, according to current knowledge, the QoL of patients with DH seems to be reduced, but importantly, already after adherence to a GFD for 1 year, the QoL increases to the level of controls (35). The positive impact of GFD on DH patients' QoL is also supported by another study, in which the Qol of long-term GFD-treated DH patients was equal to that of controls, and slightly better than that of long-term treated coeliac patients (74).

Similar to coeliac disease, DH has been associated with other autoimmune diseases, and the associations have mostly been explained by common genetic factors. In DH, the frequency of autoimmune thyroid disease has been reported to be as high as 4% and that of type 1 diabetes 1–2% (75–78). In addition, Sjögren's syndrome, vitiligo and alopecia areata have been reported to associate with DH, although these associations are not well documented. Most of the associated autoimmune diseases have been reported to develop prior to the diagnosis of DH, but subsequent development is also a possibility. A recent Finnish register study demonstrated a rather interesting association of DH with bullous pemphigoid (79). In that study, patients with previously diagnosed DH had a 22-fold risk for the later development of bullous pemphigoid, with a mean of 3 years from diagnosis of DH to diagnosis of bullous pemphigoid. The authors speculated that a possible mechanism of this evolvement could be an epitope spreading phenomenon.

CONCLUSION

DH is a chronic, bullous skin disease, which is a skin manifestation of coeliac disease. It is suggested that long-lasting and undetected coeliac disease with TG2-directed immune response serves as a prerequisite for the development of DH and TG3 antibody response and, furthermore, that more accurate and active coeliac disease diagnostics has resulted in a declining incidence of DH (5, 6). The cutaneous symptoms of DH are troublesome and decrease the QoL of patients (35). It is therefore fortunate that the diagnostic delay has become shorter during recent decades (39). However, variable prevalence figures for DH in different countries, and delayed diagnosis in one-third of patients with DH in a high prevalence area (39) indicate that there is still a necessity for further improvement of DH diagnostics. Recognizing the cutaneous signs indicative of DH and IF examination of perilesional skin biopsy remain the cornerstones of DH diagnosis (Table I). Investigation of small bowel mucosal histology has no further value in routine diagnostics, and TG2 antibody testing has a supportive, but not exclusive, role in DH diagnosis. Future studies will presumably reveal whether measurement of TG3 antibody has additional value in DH diagnostics or in the identification of subjects at risk of development of DH. One future prospect is that TG3 antibody-based diagnosis of DH could be a possibility in the long run, which would facilitate the diagnosis of DH and enable diagnostics in centres without the possibility of IF examination. In coeliac disease, serologically-based diagnosis has been recommended in children since the year 2012 (80), and is also utilized in adults in some countries, such as Finland.

According to current knowledge, strict life-long adherence to GFD is justified in all patients with DH. The prognosis seems to be excellent in those individuals with DH who follow the diet rigorously, but other than adherence to a GFD, little is known about the factors that influence the development of complications or associated diseases of DH and mortality. Instead, it has been shown that the degree of villous atrophy has no effect on the above-mentioned outcomes of DH (57, 58). Factual non-responsiveness to GFD is rare in DH, but, in general, refractory DH seems to have better prognosis compared with refractory coeliac disease (61). However, current knowledge of refractory DH is scarce and more research evidence is needed in order to elaborate this entity more thoroughly. In addition, the differing mortality trends currently existing among DH and coeliac disease patients adhering to the same diet is an interesting topic for future studies.

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REVIEW ARTICLE

Bullous Drug Reactions

Maja MOCKENHAUPT
Dokumentationszentrum schwerer Hautreaktionen (dZh), Department of Dermatology, Medical Center and Medical Faculty, University of Freiburg, Freiburg, Germany

Bullous drug eruptions are infrequent, but because they pose a challenge both to affected patients and to treating physicians they are considered to be the most severe cutaneous adverse reactions (SCAR). It is important to recognize these conditions and to differentiate them from other clinical entities involving blister formation. There may be early signs and symptoms that indicate a severe bullous drug eruption even before blisters and erosions of the skin and mucous membranes become obvious. Once the diagnosis is suspected, appropriate diagnostic procedures and adequate management must be initiated. The latter includes identification of the potentially inducing drug, although it should be taken into account that not all cases of bullous eruptions are drug-induced. In cases with drug causality the potentially culprit agent must be withdrawn, while in cases with other aetiology the underlying condition, e.g. an infection, must be treated appropriately. In addition to best supportive care, immunomodulating therapy may be considered.

Key words: severe cutaneous adverse reaction; Stevens-Johnson syndrome; toxic epidermal necrolysis; generalized bullous fixed drug eruption.

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Corr: Maja Mockenhaupt, Dokumentationszentrum schwerer Hautreaktionen (dZh), Department of Dermatology, Medical Center and Medical Faculty, University of Freiburg, Hauptstr. 7, DE-79104 Freiburg, Germany. E-mail: dzh@uniklinik-freiburg.de

SIGNIFICANCE

Drug reactions with blisters (known as bullous drug reactions) are challenging for patients and physicians. Often there are early signs and symptoms that may lead to the suspicion of a bullous drug reaction before blisters and erosions of the skin and mucosa appear. Once the diagnosis is suspected, appropriate diagnostic and therapeutic procedures must be initiated. A detailed history, including clinical symptoms, drug use and infections, is crucial. In cases with drug causality, the potentially culprit agent must be withdrawn, while in cases with other aetiology, the underlying condition, e.g. infection, must be treated. In addition to best supportive care, immunomodulating therapy may be considered.

Bullous drug reactions generally occur as a result of medication use, but there are also other possible causes. One of the major challenges is to identify at a very early stage whether the reaction will be severe and life-threatening. Once blisters are present, differentiation between types of reaction, such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), generalized bullous fixed drug eruption (GBFDE) and, sometimes, bullous autoimmune disease, is also challenging, since, for example, conditions such as GBFDE or IgA-linear dermatosis can mimic SJS/TEN. Differentiation is important as prognosis and treatment modalities differ substantially.

HISTORICAL CONSIDERATIONS

In 1922, the American paediatricians Stevens & Johnson (1) reported 2 cases of a disseminated cutaneous eruption associated with erosive stomatitis and severe ocular involvement. In 1956, the Scottish dermatologist Lyell (2) described patients with epidermal loss secondary to necrosis, and introduced the term "toxic epidermal necrolysis". However, Lyell did not refer to the findings of Stevens & Johnson at that time, but in a later reappraisal evaluated the original 4 cases in his publication as SJS/TEN, staphylococcal scalded skin syndrome (SSSS) and generalized bullous fixed drug eruption (GBFDE) (3). The histopathological difference between an intraepidermal subcorneal separation in one case had already been described in his first publication, but it took until 1971 to identify a staphylococcal exotoxin as the cause of this reaction and to name it accordingly (4). Around the same time Kauppinen (5) from Finland separated a multilocular or GBFDE from SJS and TEN through clinical features and behaviour in allergological testing.

Over the years, due to similarities in clinical and histopathological features, SJS and TEN have been included in the spectrum of erythema multiforme (EM), which was first described by von Hebra in 1860 (6). However, several attempts have been made to disentangle, regroup and rename the reactions. Ruiz-Maldonado (7), for example, proposed the term "acute disseminated epidermal necrosis" for SJS, TEN and "transmission of forms", but did not separate EM, whereas Lyell (8) suggested the name "exanthematic necrolysis" for SJS/TEN. Based on the original descriptions and the observation that SJS may progress into TEN, an international group of dermatologists developed a consensus definition that separates these conditions from EM. Because SJS and TEN share a clinical pattern, histopathological findings, aetiology, risk factors, and mechanisms, they are considered as severity variants of a single disease entity

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that differs only in the extent of skin detachment related to the body surface area (BSA) (9). Therefore, it seems more appropriate to use the term "epidermal necrolysis" or "epithelial necrolysis" (referring to skin and mucosa) for both (10).

EPIDEMIOLOGY

Epidermal necrolysis (EN) is a rare condition with an overall incidence of 1–2 cases per million persons, estimated using strictly validated cases of a prospective population-based registry (11, 12). However, incidences as high as 5–6 cases per million per year derive from medical databases not primarily designed for epidemiological analysis of rare diseases (13). EN can occur at any age, but the risk increases with age and the highest incidence is seen in elderly persons over 65 years of age (14). The mean age of patients was 53.4 years (range 1–94 years) in a cohort of more than 2,200 patients (15). Women are more frequently affected, with a sex ratio of 0.6. Patients infected with human immunodeficiency virus (HIV) and, to a lesser degree, patients with collagen vascular disease (also called connective tissue disease, including rheumatoid arthritis, systemic lupus erythematosus, Sjögren syndrome, dermatomyositis, polymyositis, scleroderma, mixed connective tissue disease and some types of vasculitis) and cancer are at increased risk (11, 16). The overall mortality associated with EN is 22–25%, varying from approximately 10% for SJS to almost 50% for TEN (17–19). Several factors contribute to poor prognosis, such as larger extent of skin detachment, older age, and underlying comorbidity.

In contrast, the mortality for erythema (exsudativum) multiforme majus (E(E)MM; i.e. EM with mucosal involvement) is very low, affecting few individuals of older age and underlying conditions. The majority of patients are young (80% are younger than 40 years, 45% are under 18 years) and male (approximately 75%) (9, 20). The incidence of cases of severe EMM leading to hospitalization is of approximately the same order of magnitude as that of EN (SJS-TEN), with milder cases (EM minus with only skin involvement or cases with only mucosal involvement) occurring more frequently (15, 20).

To date, estimates of the incidence of GBFDE are lacking, since there are currently no population-based data. As with most types of cutaneous adverse reactions, GBFDE more frequently affects women. Of the affected patients 70% are older than 70 years and approximately 22% of patients die due to advanced age and disease severity (21).

CLINICAL FEATURES AND CLASSIFICATION

EN is characterized by erythematous skin, epidermal detachment and erosions of mucous membranes. The erythematous exanthema consists of atypical flat target lesions (these lack the typical 3-zone, target-like constellation of so-called typical target lesions seen in EM) and/or macules that frequently tend to become confluent and spread from cranial to caudal. Blisters develop on the erythema and coalesce. Usually, at least one mucous membrane is affected by erosion in addition to the skin. Fever and malaise are very common (10). The condition is classified according to the consensus definition: skin detachment of less than 10% of the BSA refers to SJS, and more than 30% of the BSA to TEN. Skin detachment between these values is defined as SJS/TEN-overlap (**Table I**, **Fig. 1**) (22). In approximately 95% of cases, haemorrhagic erosions of mucous membranes, including eyes, lips, mouth, vulva, glans penis, and sometimes also trachea, bronchi, urethra and anus, are present (**Fig. 2**). Due to the fact that the skin detachment progresses, turning a case initially thought of as SJS into TEN, and due to the fact that SJS and TEN share the same aetiology and pathogenesis, they are considered as a single disease entity of different severity (9).

Table I. Consensus definition of epidermal necrolysis (EN) (22)

Criteria	EM majus	SJS	SJS/TEN overlap	TEN with maculae	TEN on large erythema (without spots)
Skin detachment, %	< 10	< 10	10–30	> 30	> 10
Typical target lesions	+	–	–	–	–
Atypical target lesions	Raised	Flat	Flat	Flat	–
Maculae	–	+	+	+	–
Distribution	Mainly limbs	Wide-spread	Wide-spread	Wide-spread	Widespread

EM: erythema multiforme; SJS: Stevens-Johnson syndrome; TEN: toxic epidermal necrolysis.

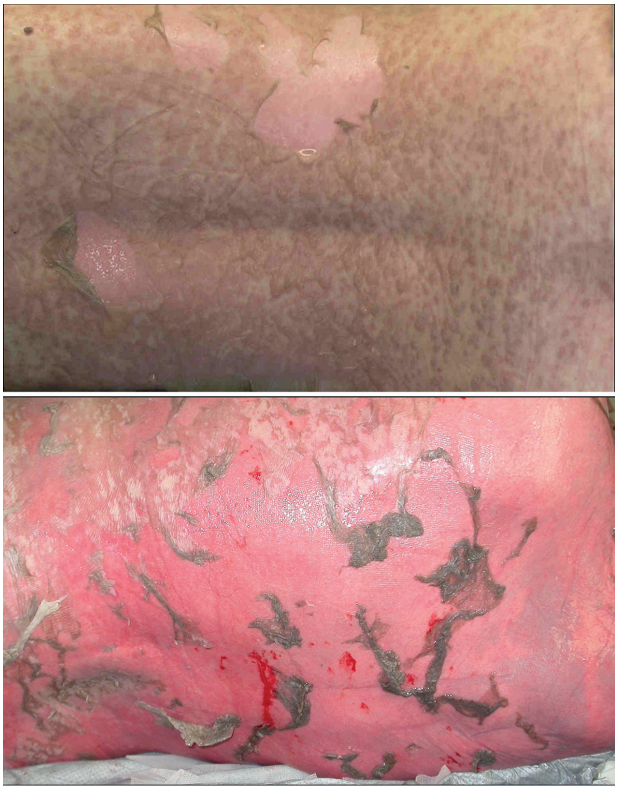

Fig. 1. Confluent macules with confluent blisters, leading to large areas of skin detachment in epidermal necrolysis (patient's back).

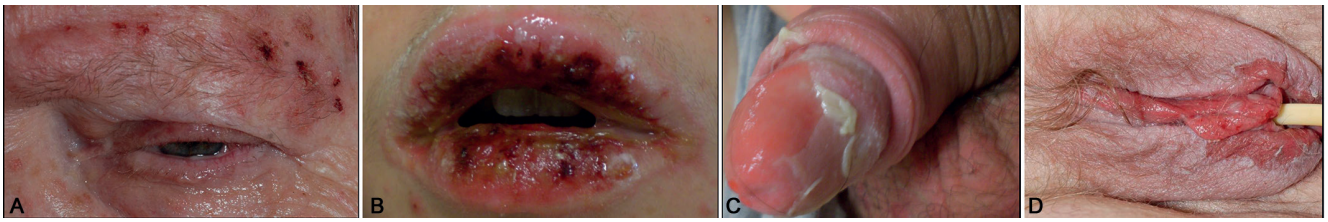

Fig. 2. Haemorrhagic erosions of mucous membranes in epidermal necrolysis or erythema multiforme majus: (a) blepharitis, (b) erosions of lips and oral mucosa, genital erosions in (c) a male and (d) a female patient.

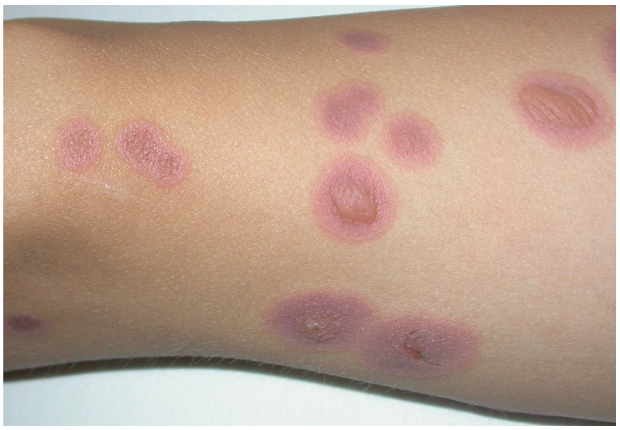

Fig. 3. Typical target lesions with central blisters in erythema multiforme majus (on the leg).

Due to the same type of mucosal involvement, EM with mucosal involvement (erythema multiforme majus; EMM) was assumed to be a less severe form of SJS. However, this incorrect classification may lead to false assessment of causal factors, which in SJS/TEN are predominantly medications and in EMM almost exclusively infections (9, 10, 20). Furthermore, younger patients with EMM may be severely ill with high fever and overall poor general state of health (20).

EMM and SJS can generally be well differentiated based on the consensus definition (in more than 90% of cases), especially when typical targets on the limbs are present (**Fig. 3**). However, differentiation may be challenging in the case of atypical EMM involving atypical "giant targets". This also accounts for the mainly truncal and generalized distribution of typical target lesions, especially in children and adolescents, since these lesions sometimes coalesce. The description of a typical and atypical type of EMM helps to better classify the various patterns of EM and their distinction from SJS (23). Moreover, due to their demarcation towards intact skin, older "giant targets" may resemble resolving patches in GBFDE (18).

Besides EMM, GBFDE is an important differential diagnosis of EN. This reaction is typically characterized by well-defined round or oval, egg-sized patches of dusky violaceous or brownish colour. Blisters may develop on these patches, but the skin remains intact between the areas of blistering and, in most cases, skin detachment does not affect more than 10% of the BSA (**Fig. 4**). However, the reaction may also present with diffuse erythema and blisters, which will show demarcation during the course. There is a debate among experts as to whether the rare cases of TEN on large erythema are potentially severe forms of GBFDE (10, 24).

Patients with GBFDE usually do not develop fever and malaise, but there may be mild mucous membrane involvement, with the genital and/or oral mucosa affected, but not the ocular surface. Milder eruptions are frequent in the patient's medical history (18, 21, 24).

To supplement the consensus definition for EN described above (22), the RegiSCAR-group developed a score for the diagnostic differentiation of GBFDE, which is currently in the validation phase and has not yet been published. There are no specific laboratory parameters to differentiate between the various types of blistering reactions.

HISTOPATHOLOGY

The histology of EN reveals necrotic (dyskeratotic or apoptotic) keratinocytes, either in a disseminated distribution or as complete epidermal necrosis with subepidermal blister formation. Localization and timing of sample collection are important: if the biopsy is taken from the central blister of an EMM target, complete epidermal necrosis may also be visible, as well as a sparse superficial lymphocytic infiltrate in the dermis, often in a perivascular location (25, 26). Therefore, histopathology can only confirm the clinical condition within the spectrum of disease but is unable to proof the specific clinical form. The same accounts for the histology of GBFDE, in which a distinction is sometimes possible in the course of the disease. If a biopsy is taken at a later stage, a deep perivascular infiltrate containing

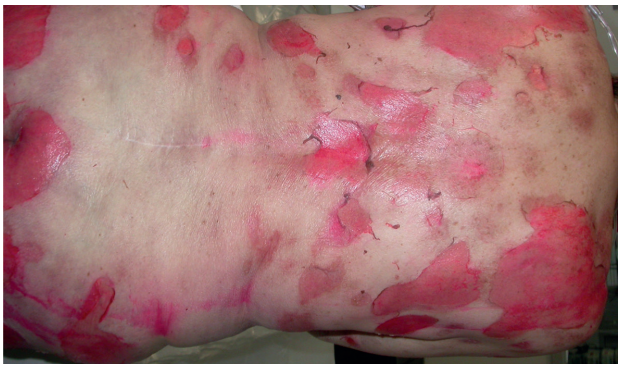

Fig. 4. Well-demarcated erythematous patches with blisters in generalized bullous fixed drug eruption (on the back).

neutrophils and eosinophils may be seen, and potentially also pigment deposits (26, 27).

FURTHER DIFFERENTIAL DIAGNOSES

EN and GBFDE must be differentiated from staphylococcal scalded skin syndrome (SSSS), which histologically shows intraepidermal, subcorneal separation (4). Bullous autoimmune dermatoses, such as bullous pemphigoid, linear IgA dermatosis, pemphigus vulgaris, and paraneoplastic pemphigus, should be included in the differential diagnosis (10). Therefore, if one of these diseases is suspected, a direct immunofluorescence test as well as serological autoimmune parameters (e.g. anti-BP 180-, 230-, desmoglein antibodies) should be performed (10). Linear IgA bullous dermatosis (LABD) can imitate SJS/TEN, as has been described in several case reports and case series (28, 29). Some authors reported a more severe pattern with larger areas of skin detachment in cases that were drug-induced, with vancomycin being a frequent cause (29). Other disorders that should be considered in the differential diagnosis include widespread drug eruptions, erythroderma, exfoliative dermatitis, and subacute cutaneous lupus erythematosus (10, 18). Acute generalized exanthematous pustulosis (AGEP) may mimic EN, when confluence of pustules appears to reveal a positive Nikolsky's sign. However, AGEP does not turn into EN, since there is no primary epidermal necrosis, but there is rapid healing of the subcorneal lesions. Bullae may occur in body areas with oedema, leading to widespread intraepidermal blister formation and secondary necrosis of the blister roof (30). If tension blisters appear due to oedema in drug reaction with eosinophilia and systemic symptoms (DRESS), EN might be suspected, but early histopathology will show that there is no full-thickness necrosis leading to epidermal detachment and that the subepidermal separation occurs first followed by secondary necrosis of epidermal cells (18). In addition, atypical target lesions on the limbs and erosions of the lips may raise the suspicion of EN, although features such as facial oedema and erythema with inflammatory infiltration of the skin point to DRESS. Therefore, it is important to monitor specific laboratory values relevant for a diagnosis of DRESS, e.g. eosinophilia, liver enzymes, kidney parameters, etc. Liver involvement, indicated by at least a 2-fold increase in transaminases, on 2 different days may occur when eosinophilia has already turned to normal values. When the skin eruption heals, widespread post-inflammatory desquamation is frequently observed and sometimes mistaken for skin detachment in EN (18, 31).

Other differential diagnoses vary with the clinical pattern and during the course of the reaction. In the early stage of the disease, maculo-papular, multiforme- or target-like drug eruptions, which can also present with oral lesions and conjunctivitis, must be considered, especially in elderly patients (**Table II**) (32). Varicella and other viral exanthems are important differential diagnoses when the first signs and symptoms occur in children (10, 18, 33).

Table II. Differential diagnoses of epidermal necrolysis (EN) (10)

Most likely
- Limited EN (SJS)
  - Erythema multiforme majus
  - Varicella
- Widespread EN (SJS/TEN overlap and TEN)
  - Acute generalized exanthematous pustulosis
  - Generalized bullous fixed drug eruption
  - Drug reaction with eosinophilia and systemic symptoms

Consider
- Paraneoplastic pemphigus
- Linear IgA bullous dermatosis
- Pressure blisters after coma
- Tension blisters due to oedema
- Phototoxic reaction
- Graft-versus-host disease
- Staphylococcal scalded skin syndrome
- Thermal burns
- Skin necrosis from disseminated intravascular coagulation or
- Chemical toxicity (e.g. colchicine intoxication, methotrexate overdose)

CLINICAL COURSE OF EPIDERMAL NECROLYSIS

EN typically begins with unspecific prodromal symptoms, such as sore throat, runny nose, cough, headache, fever, and malaise, preceding mucocutaneous lesions by 1–3 days. These symptoms are followed by the appearance of erythematous macules and atypical targets of the skin that may be confluent and on which blisters occur. Burning or stinging of the eyes, and pain when swallowing or urinating, develop progressively, heralding mucous membrane involvement. Most reactions start with non-specific symptoms, followed either by cutaneous or mucosal involvement, but some may begin directly with specific lesions of the skin and mucous membranes. The rapid progression of such symptoms, the addition of new signs, severe pain, and rapid decline in the patient's general state of health should prompt the suspicion of a severe disease (10, 33).

In the majority of EN-cases the eruption initially shows a symmetrical distribution on the face, the upper trunk, and the proximal parts of the limbs. The distal parts of the arms and legs are often spared, but the eruption may extend rapidly to the entire body within a few days or even within a couple of hours. The initial skin lesions are characterized by erythematous, dusky-red, irregularly-shaped, purpuric macules, which coalesce progressively (Fig. 1). Atypical target lesions with dark centres are often observed. Confluence of necrotic lesions leads to extensive erythema, and Nikolsky's sign (dislodgement of the epidermis by lateral pressure) is positive on erythematous areas. Flaccid blisters that burst easily are present at this stage, and the necrotic epidermis is easily detached at pressure points or by frictional trauma, revealing large areas of exposed, red, sometimes oozing dermis, whereas the epidermis may remain in other areas (10, 15, 18, 33).

In terms of severity, cases are classified according to the consensus definition (Table I) based on the total area in which the epidermis is detached or detachable (positive Nikolsky's sign). Correct evaluation of the extent of detachment is difficult, especially in areas with spotty lesions and small blisters. Therefore, it may be helpful to remember that the surface area that can be covered by one hand (the

patient's hand in children) represents approximately 1% of the patient's BSA (10, 15).

Mucous membrane involvement (in most cases on at least 2 sites) is observed in approximately 90% of patients (Fig. 2). It typically begins with erythema, followed by painful erosions of the oral, ocular, genital, nasal, anal and, sometimes, tracheal or bronchial mucosa. These symptoms usually lead to impaired alimentation, photophobia, conjunctivitis and painful urination. The oral cavity is almost invariably affected and reveals painful haemorrhagic erosions, often with greyish white pseudomembranes. The lips are covered with haemorrhagic crusts. Approximately 80% of patients have conjunctival lesions accompanied by pain, photophobia, lacrimation, redness and discharge. Severe forms may lead to epithelial defect and corneal ulceration, anterior uveitis, and purulent conjunctivitis and blepharitis. Synechiae often occur between eyelids and conjunctiva, and eyelashes may be shed. Genital erosions are frequent in men and women, but may be more easily overlooked in females, especially in young girls.

To detect such distinct features requires a thorough clinical examination of the patient's entire body, involving further specialists in the examination of eyes, deep throat and genital mucosa in women. Ophthalmological consultation, in particular, is an urgent requirement to prevent complications and long-lasting sequelae (10, 15, 18, 33).

AETIOLOGY AND MEDICATION RISK

Although more than 100 different drugs have been reported in the literature as inducers of EN, less than a dozen have been identified to carry a high risk, and these account for more than half of the cases occurring in Europe according to 2 multinational case-control studies (16, 34). These high-risk drugs are allopurinol, antibacterial sulphonamides, certain antiepileptic drugs, such as carbamazepine, lamotrigine, phenobarbital and phenytoin, non-steroidal anti-inflammatory drugs (NSAIDs) of the oxicam-type, and nevirapine. The risk appears to be confined to the first 8 weeks of treatment and most reported EN cases started after the first continuous use of the medication between 4 and 28 days (16, 34, 35, 36). For lamotrigine and the anti-HIV-drug nevirapine, it was thought that a slow titration of the dosage could prevent such severe adverse reactions, since slow dose escalation had been shown to decrease the rate of mild eruptions. However, there is no evidence for a decreasing risk of EN (37–39). Oxcarbazepine, a 10-keto derivative of carbamazepine, which was considered to have a far lower risk, seems to cross-react with carbamazepine, revealing a lower, but substantial, risk of causing EN. Allopurinol, an old drug used to treat hyperuricaemia and gout, is widely believed to be a very safe medication; however, it was identified as the major cause of EN in Europe and Israel more than a decade ago and remains as such to date (40, 41).

Often the entire group of NSAIDs is suspected to induce EN, but there is a huge difference in risk among the various groups: oxicam derivatives carry the highest risk, acetic acid derivatives (e.g. diclofenac) moderate risk, and propionic acid derivatives (e.g. ibuprofen) no increased risk (**Table III**) (34, 36).

Among anti-infective agents, a significant, but much lower, risk than for antibacterial sulphonamides has been shown for different groups of antibiotics, such as cephalosporins, quinolones, tetracyclines and aminopenicillins. For other medications, such as corticosteroids, proton pump inhibitors or tramadol, the calculated risk was

Table III. Drugs and recommendations in epidermal necrolysis (EN) (34)

A. Drugs with a high risk of inducing EN

Use of these drugs should be evaluated carefully and they should be suspected promptly.

- Allopurinol
- Carbamazepine
- Co-trimoxazole (and other anti-infective sulphonamides and sulfasalazine)
- Lamotrigine
- Nevirapine
- NSAIDs (oxicam type, e.g. meloxicam)
- Phenobarbital
- Phenytoin

An interval of 4–28 days between start of drug use and onset of adverse reaction is most suggestive of an association between the medication and SJS/TEN.

When patients are exposed to several medications with high expected benefits, the timing of administration is important to determine which one(s) must be stopped and if some may be continued or re-introduced.

The risks of various antibiotics to induce EN are within the same order of magnitude, but substantially lower than the risk of anti-infective sulphonamides.

B. Drugs with a moderate (significant but substantially lower) risk of EN

- Cephalosporins
- Macrolides
- Quinolones
- Tetracyclines
- NSAIDs (acetic acid type, e.g. diclofenac)

C. Drugs with no increased risk of EN

- Beta-blockers
- ACE inhibitors
- Calcium channel blockers
- Thiazide diuretics (with sulphonamide structure)
- Sulfonylurea anti-diabetics (with sulphonamide structure)
- Insulin
- NSAIDs (propionic acid type, e.g. ibuprofen)
- Valproic acid

NSAIDs: non-steroidal anti-inflammatory drugs; ACE inhibitors: angiotensin-converting-enzyme inhibitors.

strongly affected by confounding (16, 34). In comparison with the results of 2 case-control studies, recent analysis of systematically ascertained registry data on EN using ALDEN (algorithm for causality assessment in EN) (42) demonstrated that the proportion of validated cases that could be explained by medications with a significant (high and moderate) risk was stable (65–68%) over a period of more than 2 decades (16, 34, 42). ALDEN provides structured help for identifying the most likely culprit drug and is based on the following criteria: time latency between start of drug use and index-day (i.e. onset of the adverse reaction), drug present in the body before index-day (taking into account the drug's half-life as well as the patient's liver and kidney function), information on prechallenge/rechallenge and dechallenge (if available), type of drug/notoriety (based on drug lists that require a regular update) and alternative causes. Numerical score values lead to a causality assessment for each individual drug a patient has taken or was administered, ranging from "very unlikely", "unlikely", "possible", "probable" to "very probable (43). For approximately one-third of cases of EN, no patent drug cause could be identified by using 2 completely different epidemiological methods. Even if new drugs or combinations of old drugs are taken into account as triggers of EN, at least 25% of all cases remain without a plausible drug cause, whereas this proportion reaches 50% among children and adolescents with EN. In these cases other eliciting factors must be saught:

An important non-drug risk factor is infections within one month before reaction onset. Most often these infections are diagnosed by clinical means, but positive serology related to certain well-known infectious agents, such as Epstein-Barr virus, cytomegalovirus, adenovirus or *Mycoplasma pneumoniae*, are rare. In some cases a preceding infection cannot be distinguished from the prodromal symptoms of EN; in others the reaction occurs suddenly with no prior signs or symptoms and must be labelled as "idiopathic" (10, 24).

EN has also been reported in the context of bone marrow transplantation, some eruptions of which may be induced by medication use, others are rather a maximal variant of acute graft-versus-host disease (GVHD). However, clinical and histological findings in EN and extensive acute GVHD are often indistinguishable, but depending on reaction onset after transplantation and the presence of non-cutaneous symptoms of GVHD, this diagnosis seems to be more likely (44). Lupus erythematosus (systemic LE or subacute cutaneous LE) is associated with an increased risk of EN. Often drug causality is doubtful in such cases and keratinocyte necrosis and subsequent skin detachment may be an extreme phenotype of cutaneous LE that must be considered as a differential diagnosis of EN (45).

For drug analysis in epidemiological studies, as well as for causality assessment in an individual case of EN, the correct determination of the day of reaction onset (so-called index-day) is of major importance (10, 15, 33, 34). All medications taken within a month preceding the index-day should be listed with their first and last day of use. Furthermore, information on prior use is very important, since it is rather unlikely for a medication to be the cause of EN if it was taken and tolerated in the past. A drug inducing EN is typically taken as the first continuous use, most often for 1–4 weeks, but sometimes for up to 8 weeks, without prior exposure (34). Thus, the mechanism differs from the classical sensitization in allergic conditions (10, 15, 37, 38, 39).

Frequently, and especially when no obvious drug cause is identifiable, medications taken to treat the prodromal symptoms are suspected of having induced the reaction. This mainly concerns antipyretics, analgesics, and secretolytics, sometimes summarized as "cough and cold medicines." When looking more closely at the use of these medications, they have usually been taken and tolerated previously and/or were started after the onset of prodromal symptoms of EN ("protopathic bias"). Neither of these patterns is typical for drug exposure causing EN (15, 33, 46). In contrast, medications causing EN have not been used previously and their exposure represents the first continuous use that started 4 weeks to at least 4 days before reaction onset. Furthermore, these substances do not belong to the drug groups for which an increased risk was estimated in epidemiological studies (34, 35). Differentiation between infection and drugs as the triggering agent can be challenging in the case of antibiotics used to treat infections ("confounding by indication"), but it helps to consider the type of infection, since classic bacterial infections alone do not seem to have an increased risk of causing EN (33).

For GBFDE, there are numerous case reports in the literature providing information on possible drug triggers (47–50); however, no analyses have been conducted on large patient numbers. The range of triggers include antimicrobial sulphonamides (especially cotrimoxazole), analgesics (especially metamizole, but also paracetamol), and, less frequently, antibiotics, allopurinol, and antiepileptic drugs (especially carbamazepine) (47–50). The latency between the start of drug use and reaction onset ranges from a few hours to a few days. In contrast to EN, the triggering agent has often been used and tolerated in the past (18). Sensitization happens over time, meaning that a reaction consistent with a fixed drug eruption occurs rapidly upon renewed use of the drug. Thus, GBFDE is a classic allergic reaction that must be differentiated from EN.

RISK OF RECURRENCE

The risk of recurrence in EN appears to be rather low, as Kirsti Kauppinen had already observed in 1972 (5). In the multinational RegiSCAR study, few individual patients experienced a second event of EN after accidental exposure to the same drug that had induced the first event. The time latency between the start of drug use and reaction onset was very similar and not necessarily shorter, as reported repeatedly in the literature.

In contrast, fixed drug eruption, including GBFDE, has a high risk of recurrence, which may be explained by memory T cells remaining in the affected skin (51). In many cases there has been a previous, often less severe, event, but cases

with extensive skin detachment may also occur *de novo* and re-occur with the same amount of involvement (49).

EMM appears to be almost exclusively triggered by infections, especially *M. pneumoniae* in children and adolescents, and herpes simplex virus in adults. Recurrence has been observed, in up to 10% of cases, and in some patients even several times, before the reaction resolves (20). Interestingly, infection-induced EN cases do not seem to recur, and it may be assumed that the viral triggers change so rapidly that they are not recognized again as an antigen (52).

PATHOGENESIS AND GENETICS

A T-cell reaction comparable to GVHD is believed to be the pathogenetic mechanism in EN, since immunohistochemical investigations identified primarily $CD4^+$ cells in the dermis and $CD8^+$ cells in the epidermis (53, 54). In contrast to what was postulated in earlier years, these cytotoxic T cells are usually specifically directed against the native form of the drug rather than against reactive metabolites (55). The acute necrosis of keratinocytes in EN is attributed to an extensive process of apoptosis (54, 56). Cytotoxic T-cells are able to initiate apoptosis, enhanced by the release of perforin and cytokines, such as TNF-α or granzyme B (57, 58). It is also assumed that proteins such as Fas antigen (CD 95) and the P55 TNF-α receptor enhance apoptosis in keratinocytes (59). However, it was demonstrated that Fas and Fas ligand are not the most important cytokines in the acute phase of EN, but rather the cationic protein granulysin (60). It showed the strongest cytotoxicity in the blister fluids of patients with EN compared with other blistering diseases, with its concentration correlating with the severity of the clinical reaction (60). Therefore, it was concluded that granulysin is a severity marker in EN and provides a target for possible immunomodulating treatments. It has also been shown that IL-15 is associated with the severity of the reaction as well as the risk of mortality (61).

It has been known for many years that there is a genetic predisposition to develop EN. As early as 1987, different human leukocyte antigen (HLA) loci were found for TEN associated with sulphonamides or with oxicam-NSAIDs (62). Almost 20 years later, a strong association between HLA-B*1502 and carbamazepine was observed in patients with EN who were of Han Chinese descent (63). This association could not be detected in European patients, where HLA-B*5701 was identified to confer genetic susceptibility to carbamazepine-induced SJS/TEN (64). Interestingly, HLA-B*5701 had previously been demonstrated to be associated with abacavir hypersensitivity, which is characterized by fever, rash and constitutional, gastrointestinal, and/or pulmonary symptoms different from SJS/TEN and DRESS (52). A second strong association with HLA-B*5801 was observed in Han Chinese patients with allopurinol-induced disease, not only for EN, but also for DRESS (65). For this allele an association of 55% was found in allopurinol-induced EN cases of European descent (66). Clearly, genetic predisposition is not the only important factor for developing a certain type of severe cutaneous adverse reaction due to a specific drug, but also the patient's ethnicity, as was shown for patients of southeast Asian, European and African descent (52).

To date, there have been no systematic investigations into the genetic pattern of infection-induced EN cases. However, some reports on specific HLA alleles in cases thought to be triggered by antipyretics and secretolytics appear to be ultimately associated with infection-induced reactions (46). Although a large genome-wide association study in European patients with EN demonstrated that the relevant alleles/genetic variants are all located in the HLA locus on chromosome 6, the variability in the European population appears to be too large to deploy a medication-specific predictive test to prevent EN (67). In contrast, this has been successfully demonstrated in Southeast Asian subjects, at least in the case of carbamazepine, for which the predictive test has led to a marked reduction in carbamazepine-induced EN cases (68).

Although no systematic investigations into the pathogenesis of GBFDE have yet been undertaken, there are analyses on the T-cell population in fixed drug eruption. T cells play an important role here, since they remain in the affected areas of skin as "memory cells", which explains why a reaction re-occurs at the same site. The term "fixed drug eruption" takes this fact into account, although the reaction may expand if it recurs (51). Furthermore, several cytokines, such as FAS/FAS-L, perforin and granzyme B, are equally expressed in GBFDE and EN, whereas the concentration of granulysin is much lower in GBFDE compared with EN (27).

THERAPY

Taking a detailed and thorough medication history is crucial. Assuming a medication rather than an infection triggered the reaction, the most likely culprit drug should be identified and discontinued. Thus, it is essential to know the time latency between the start of drug use and onset of the reaction, as well as the drugs that have a high-to-moderate risk of the type of reaction in question. It may be helpful to create a timeline diagram, into which the chronological sequence of clinical symptoms is entered on the x-axis and the medications taken or applied are entered on the y-axis (**Fig. 5**). Based on the diagram and the information on duration of use (start and end of use), it is possible to narrow down or even identify the inducing agent. It then becomes obvious that not all drugs, some of which may be vital for life, need to be withdrawn. Medications that were administered to treat prodromal symptoms and that are often suspected as the cause of EN, can also be excluded as triggers. If an infection is thought to have induced the reaction, patients should receive adequate antibiotic or antimicrobial treatment; reluctance to provide medication

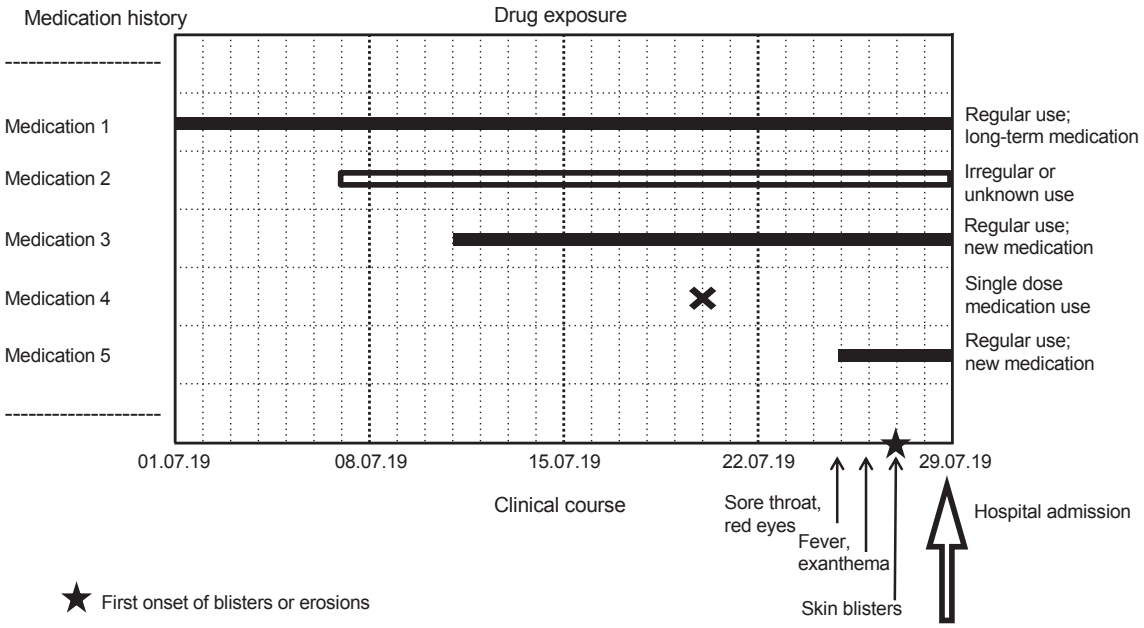

Fig. 5. Timeline diagram with chronologic sequence of clinical symptoms (x-axis) and medication use (y-axis).

in a medical condition frequently caused by drugs may be detrimental (15, 33). The following supportive care and topical treatment is recommended:

In order to assess a patient's prognosis and to decide on the appropriate therapeutic options, the SCORTEN (severity-of-illness score for EN) has been developed (69). Seven independent, but equally significant, factors are used for the calculation of score points: (*i*) age (≥40 years), (*ii*) heart rate (≥120/min), (*iii*) malignancy, (*iv*) percentage of detachment relative to BSA on day 1 (≥10 %), (*v*) serum urea (>10 mmol/l), (*vi*) serum bicarbonate (<20 mmol/l), and (*vii*) serum glucose (>14 mmol/l) (69). The positive score points are added and the higher the value, the higher is the risk of death and the lower the chance of survival (69–71) (**Table IV**).

Only patients with limited skin involvement, and a SCORTEN value of 0 or 1, and a disease that is not rapidly progressing can be treated in non-specialized wards. Depending on the local or national facilities, patients who do not need intensive care may remain in dermatology units or hospitals (in many European countries), others should be transferred to intensive care facilities or burn units (72, 73). Supportive care is still the cornerstone of treatment and includes maintaining haemodynamic equilibrium and preventing life-threatening complications. Due to significant fluid loss in patients with large amount of skin detachment, hypovolaemia and electrolyte imbalance must be adjusted on a daily basis. Infusion volumes are usually lower than for burns of a similar extent of skin detachment (approximately 1/3–/4 of the infusion volume in burns) because interstitial oedema is absent. In order to select the correct amount of fluid replacement, correct estimation of the denuded BSA is important (74). Peripheral venous lines should be used, if possible, since the sites of insertion of central lines are far more prone to infection. Increasing the environmental temperature to 25–30°C is important to compensate for loss of thermoregulation in patients with extensive skin detachment (72). Air-fluidized beds may help to increase the patient's comfort. To reduce the risk of infection, aseptic and careful handling is required. Skin, blood, and urine specimens should be cultured for bacteria and fungi at frequent intervals. Prophylactic use of antibiotics should be avoided, and instead patients with EN should receive antibiotics when an infection is suspected based on clinical features and laboratory results. Prophylactic anticoagulation is needed and early nutritional support should be provided through nasogastric tubes in order to promote healing and decrease the risk of bacterial translocation from the gastrointestinal tract (10, 18, 72, 73). For adequate enteral nutrition, intensive care guidelines (e.g. ESPEN guidelines) should be followed (75).

Table IV. SCORTEN (69) (severity-of-illness score for epidermal necrolysis) to assess a patient's prognosis

Factor	Score	Weight/score value
Age, years	≥40	1
Malignancy	Yes	1
Body surface area detached (day 1), %	≥10	1
Tachycardia, /min	≥120	1
Serum urea, mmol/l	≥10	1
Serum glucose, mmol/l	≥14	1
Serum bicarbonate, mmol/l	<20	1
Possible score		0–7

Topical treatment plays a special role in bullous reactions. Antiseptic solutions or gels, as well as non-medicated and non-adhesive gauze dressings are used. There is no standard policy concerning the use of antiseptics and wound dressings, which remains a matter of experience in each centre. Careful handling and skilful wound care, performed by experiences nurses, in addition to adequate pain management, are essential (10, 72, 73).

Some experts recommend leaving the blister roof in place as a natural cover to protect the dermis, while others recommend complete removal of detached skin and the consecutive use of biosynthetic dressings in order to protect against infection. Although this remains a topic of debate, it was recently suggested that aggressive debridement is neither necessary in superficial burns nor in EN, because superficial necrosis is not an obstacle to re-epithelialization and might even accelerate the proliferation of stem cells due to inflammatory cytokines (76).

In the case of erosive mucous membrane involvement, local antiseptic treatment is recommended and the appropriate medical specialist should be consulted. In terms of eye involvement, an experienced ophthalmologist should examine the patient immediately after admission. Preservative-free emollients, antibiotic or antiseptic eye drops, often alternating with anti-inflammatory (e.g. corticosteroid) eye drops are recommen-

ded every 2 h in the acute phase. In case of early synechiae, mechanical disruption is indicated and graft of cryopreserved amniotic membrane has been proposed to decrease the rate of severe ocular sequelae. In any case, severe ocular involvement requires daily consultation with an ophthalmologist (73, 77).

Disinfectant mouthwash can be used for treatment of oral erosions, whereas erosions of the lips should be treated with bland ointment, e.g. dexpanthenol. Genital erosions in male and female patients may lead to adhesions or strictures. To avoid such complications wet dressings or a sitz bath are helpful. If deeper vaginal involvement is suspected in young girls, a gynaecological examination should also be performed, since early adhesions must be carefully disrupted. To avoid these, dilators covered with ointment can be applied (78).

Since GBFDE is considered to be a self-limiting disease that ceases to progress shortly after discontinuation of the triggering drug, supportive care alone is adequate. However, complications requiring intensive care can occur, especially in older patients and patients with extensive skin detachment. Topical treatment is the same as in EN. Since the mucous membranes are most often unaffected, interdisciplinary consultations are not mandatory, but can be helpful in some cases (24, 48).

Immunomodulating treatment. Because of the immunological mechanisms with involvement of cytotoxic T-cells and release of cytokines, several immunosuppressive and anti-inflammatory treatments have been tried to halt the progression of the disease. Data on therapeutic approaches largely derive from uncontrolled case series and case reports. Due to the rarity of SJS/TEN and the resulting low patient numbers, as well as the unexpected onset and rapid progression of the reaction, it remains a huge challenge to conduct a controlled randomized study on treatment efficacy. Therefore, existing data on treatment of EN must be evaluated with care:

- Glucocorticosteroids are the most frequently used immunomodulating treatment in patients with EN (18), but their use is controversial, since they may increase the risk of infection and septicaemia and delay wound healing (79). However, a recently published meta-analysis on the treatment of EN that investigated publications in the period 1990–2012 demonstrated that the administration of systemic glucocorticosteroids conferred a survival benefit compared with supportive care alone (odds ratio (OR) 0.54; 95% confidence interval (95% CI) 0.29–1.01) (80). A number of smaller case series on the administration of glucocorticosteroid pulse therapy with methylprednisolone or dexamethasone (100 mg/day for 3 days) demonstrated a benefit when comparing the expected number of deaths by SCORTEN with the actually observed death rate (81, 82). A case series of 5 patients reported on the positive effect of methylprednisolone pulse therapy (500 mg/day for 3 days) in massive eye involvement on the development of ocular sequelae; this effect could not be confirmed in larger observational studies (82, 83). Thus, individual case reports and small case series should be viewed with caution. Nevertheless, if administered short-term at a medium dose (50–250 mg) for only a few days, glucocorticosteroids are a treatment option with a positive effect on swollen and painful mucous membranes, but little impact on the progression of skin detachment (80, 84).
- Intravenous immunoglobulins (IVIG) have been suggested as therapy option based on the assumption that Fas-induced keratinocyte apoptosis is blocked by antibodies present in human IVIG (85). Their use remains a subject of controversy, given that some reports described a positive effect (85, 86), whereas others were unable to show any benefit (80, 84, 87, 88). However, a number of methodological weaknesses and problems were found in the studies showing a positive effect for IVIG (89). Furthermore, the effect of IVIG dose is often the focus of the discussion. In studies that showed a disadvantage for IVIG, the dose was mostly ≤2 g/kg BW, whereas it was at least 2.8 g/kg BW in positive studies (88). Nevertheless, using SCORTEN for comparison, a more recent retrospective study of 64 patients revealed that the administration of IVIG did not have a positive effect on survival, not even at a higher dose (90). Two extensive meta-analyses also found no survival benefit for patients with EN who received treatment with IVIG compared with supportive therapy (80, 91).
- Cyclosporine A has strong immunomodulating capacity and thus has been used in the treatment of EN. Its mechanism may, on the one hand, be activation of T-helper cells and cytokines, and, on the other hand, inhibition of CD8+ cytotoxic mechanisms followed by an anti-apoptotic effect of several cytokines. The first larger retrospective case series, in which 11 patients were treated with 2×3 mg/kg BW/day, was published as early as 2000 (92). The progression in skin detachment stopped and wound healing was faster in the patient group receiving cyclosporine A compared with the control group, which received cyclophosphamide and glucocorticosteroids (92). In the following years, individual case reports and case series were published, all showing a survival benefit in patients treated with cyclosporine A compared with SCORTEN values and/or other systemic therapies (93–95). A recent larger study was conducted in Madrid and used 3 different approaches to assess the effect of cyclosporine A. Again, re-epithelialization began earlier than in the comparison group (IVIG, glucocorticosteroids, supportive care only), and the observed mortality was lower than expected by application of SCORTEN, whereas in the comparison group more patients than estimated died (96). Children and adolescents were not included in many of these studies, but cyclosporine A has been used successfully in children with EN in smaller case series (97). The 2 meta-analyses mentioned above concluded that cyclosporine A is a very promising treatment, because first, re-epithelialization begins earlier and, second the observed mortality is lower than expected (80, 91). The recommended dose is 3–5 mg/kg BW/day for a total of 10 days, but adjustment of the dose may be needed in patients with impaired renal function (98). Therefore, it is necessary to monitor creatinine levels during treatment. Close surveillance of creatinine levels is advisable in the case of higher doses and renal insufficiency, but not necessarily mandatory in other cases. Strict contraindications to short-term treatment with cyclosporine A at the suggested doses are rare, but there are only a few reports on the treatment of elderly patients (>70 years) with EN (98).
- TNF-α inhibitors have also been tried for treatment of EN, since elevated TNF-α levels were found in blister fluids, serum, and skin samples of patients with EN, and the level correlated with the severity of the reaction (99, 100). Therefore, the use of TNF-α inhibitors appeared as a potential treatment approach in EN. In 1998, a randomized double-blind placebo-controlled treatment study using thalidomide in patients with EN was terminated early, because significantly more patients in the thalidomide group died than in the placebo group (100). Paradoxical high levels of TNF-α were detected in the serum of patients in the treatment arm of the study. However, later studies used other TNF-α inhibitors, e.g. infliximab and etanercept, for the treatment of EN, but only scant reports of treatment success have been published (101, 102). In a randomized treatment study that was published recently a lower mortality in patients with EN treated with etanercept compared with the achieved SCORTEN values was observed. Wound healing started earlier and the inhibitor reduced the levels of

TNF-α and granulysin in serum and blister fluids compared with the glucocorticosteroid-treated control group (103). The prospective randomized study design can be regarded positively, since treatment studies of that kind are lacking in the area of severe skin reactions. However, most results are not significant and this study also had a number of methodological problems. The delayed re-epithelialization in the control group could be due to the prolonged use of corticosteroids.

- *Other immunomodulatory treatment options*. Other therapies have been used to treat EN, but the reliability of the findings is very low due to the small number of patients treated. In some cases, these options are no longer, or only rarely, used, as in the case of cyclophosphamide (80). Other treatments, such as plasmapheresis, which is based on the removal of cytokines involved in apoptosis, are still used, although they were not able to demonstrate verifiable positive results (104, 105).

To date, there are no data from clinical trials on the benefit of systemic immunomodulating therapy in the treatment of GBFDE. Systemic glucocorticosteroids are also used in some patients, but it appears that their short-term use does no harm and does not result in faster healing (18, 49).

COMPLICATIONS AND SEQUELAE

During the acute stage of the disease, EN may be accompanied by hepatitis, tubular nephritis, or tracheobronchial mucosal involvement, which usually resolve rather quickly (10, 73). The most common complications include nosocomial infection and septicaemia, frequently caused by central venous catheters. Therefore, peripheral catheters should be preferred wherever possible and specific hygiene measures are advised, e.g. reverse isolation, etc. (72, 73).

The majority of EN survivors experience long-term sequelae of varying severity, affecting primarily the skin and mucous membranes (106, 107). Whereas skin lesions generally heal without scarring, hyper- and hypo-pigmentation of the skin as a result of the inflammatory reaction often persist for months to years. Reversible loss of hair and nails, as well as nail growth disorders are frequently observed. Mucosal adhesions that may cause strictures in, for example, the urethra or oesophagus, represent a greater problem. By far the most hazardous and, for the patient, most dramatic, sequelae affect the eyes by symblepharon formation with entropium and trichiasis, which can even cause blindness (10, 15, 77, 106, 107).

Many patients still experience somatic as well as psychological sequelae years after their reaction. These sequelae may range from symptoms of post-traumatic stress, sleep disorders, and nightmares, to fear of using any medications. A large survey, performed 5 years after EN, revealed that many patients and their relatives are inadequately informed about their reaction, its sequelae, and how to deal with these in the long term (107).

ALLERGY WORK-UP

EN is not an allergic reaction in the strict sense, since there is no classic sensitization as in other delayed hypersensitivity reactions. In the latter, initial use of the substance is well tolerated, with a reaction developing only upon renewed exposure. EN differs in that it typically occurs during the first course of treatment with a drug (34).

GBFDE, on the other hand, is a true allergic reaction, since previous exposure to the triggering drug has usually occurred, and repeated use often causes localized fixed drug eruptions. While renewed administration of a triggering drug in patients with GBFDE can be expected to cause a rapid onset, and possibly even more extensive, repeated reaction, EN was rarely observed following similar re-exposure (5).

Skin tests, such as the patch test, are generally safe, but most often are not helpful for confirming the suspected trigger in EN. The success of testing depends, to a great extent, on the type of reaction and the T-cell populations involved, as well as on the drug to be tested. In a study performed a few years ago in France, for example, the triggering agent was confirmed by patch testing in less than 25% of patients with EN (108). One should also bear in mind that allopurinol, a very common trigger of EN, is not suitable for skin testing due to the lack of lipophilicity and skin penetration (108, 109).

In vitro tests were the most suitable instrument to identify the inducing agent in bullous drug reactions; however, their use is yet not part of routine diagnostics and remains rather experimental. This may, in part, be due to the fact that the specificity of the various tests, e.g. the lymphocyte proliferation test, the lymphocyte stimulation test, and cytokine assays, is high, while their sensitivity is much lower (109).

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